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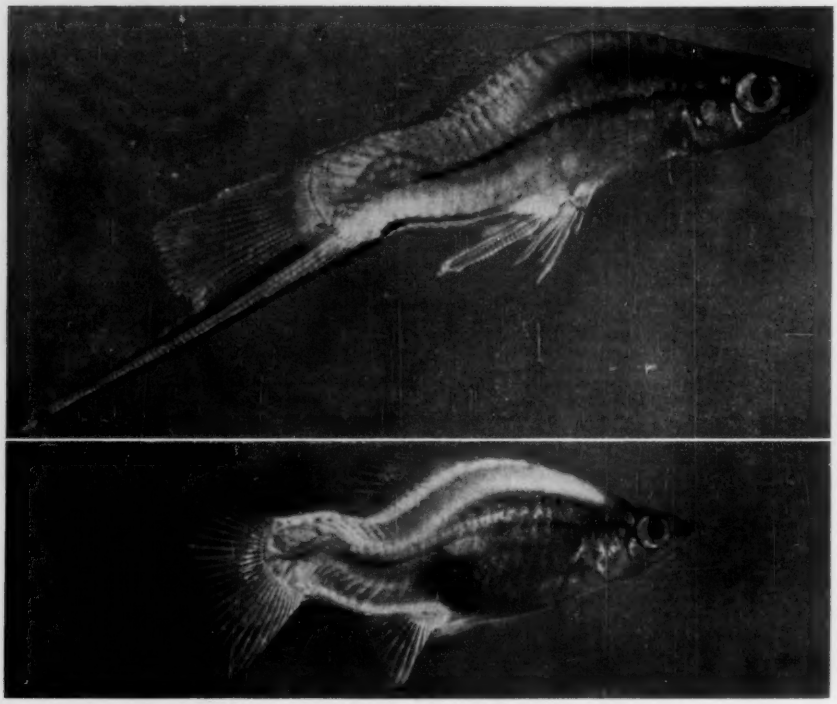

SPINAL CURVATURE IN SWORDTAIL FISH
(SEE PAGE 238)

Doddler Cattle

Sex Ratio in Mice

Polyploid Easter Lilies

Sex Chromatin of Swine Intersexes

VOLUME 49 SEPTEMBER-OCTOBER, 1958 NUMBER 5

The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks

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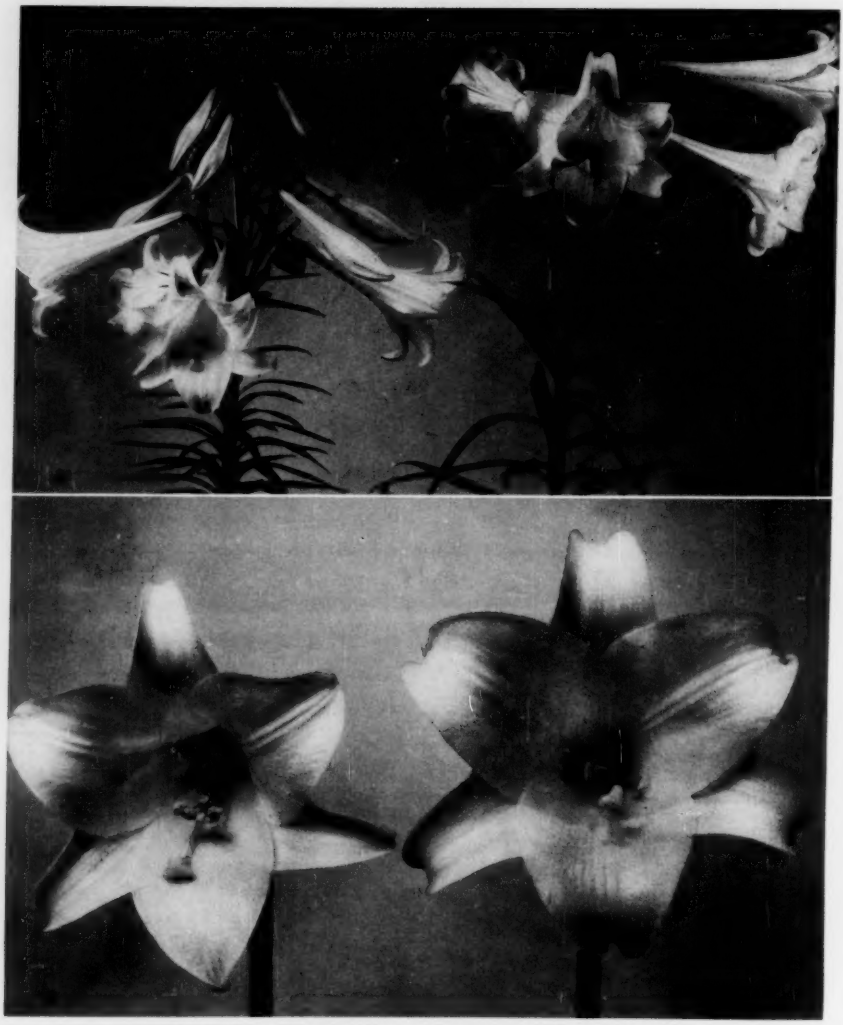


COMPARISON OF DIPLOID AND TRIPLOID SEEDLINGS

Figure 1

A and *B*—The flowering spikes and individual flowers of a diploid seedling, 54015-1 (left) and a sister triploid seedling, 54015-18 (right). The flowers of the triploid are larger and heavier-textured than those of the corresponding diploid.

THE PRODUCTION OF POLYPLOID EASTER LILIES

I. Triploids

YONEO SAGAWA*

THE object of this investigation has been the production of polyploid forms, especially triploids, of the Easter lily, *Lilium longiflorum*, which would compare favorably with some of the polyploids found among other groups of cultivated ornamental plants[17]. Although tetraploid Easter lilies have been produced by induction with colchicine[4,5,6], the plants which produce flowers that are larger and heavier-textured than the diploid are, unfortunately, frequently slower-growing, and bear fewer flowers which tend to be somewhat coarse. Among the approximately 85 species of the genus *Lilium*[21], only one natural polyploid has been reported[11,19,20]. This is the common form of *Lilium tigrinum* which has 36 somatic chromosomes ($x = 12$), making it a triploid. This triploid has larger, heavier-textured flowers than the diploid form of the species. Although self-sterile, it is distributed over a widespread area and maintains itself through the dissemination of bulbils which occur in the axils of the leaves. Among many ornamental plants, triploids have been found to possess the best array of qualities[3,8,10,12,13,14,16,22]; that is, they are intermediate in growth habit when compared to corresponding diploids and tetraploids, are floriferous and have flowers which have more substance and are larger than the corresponding diploids, but not quite as large and coarse as the corresponding tetraploids.

Materials and Methods

The diploid stud plants were the commercial clones Creole, Croft, Estate and a seedling, USDA 44, from a cross between Creole and Croft developed by the United States Department of Agriculture. In addition, a plant designated as LWQ Australian 38, was selected from a group of seedlings which presumably came from Australia. This plant has characteristics much the same as the White Queen strain offered in the trade for a garden lily.

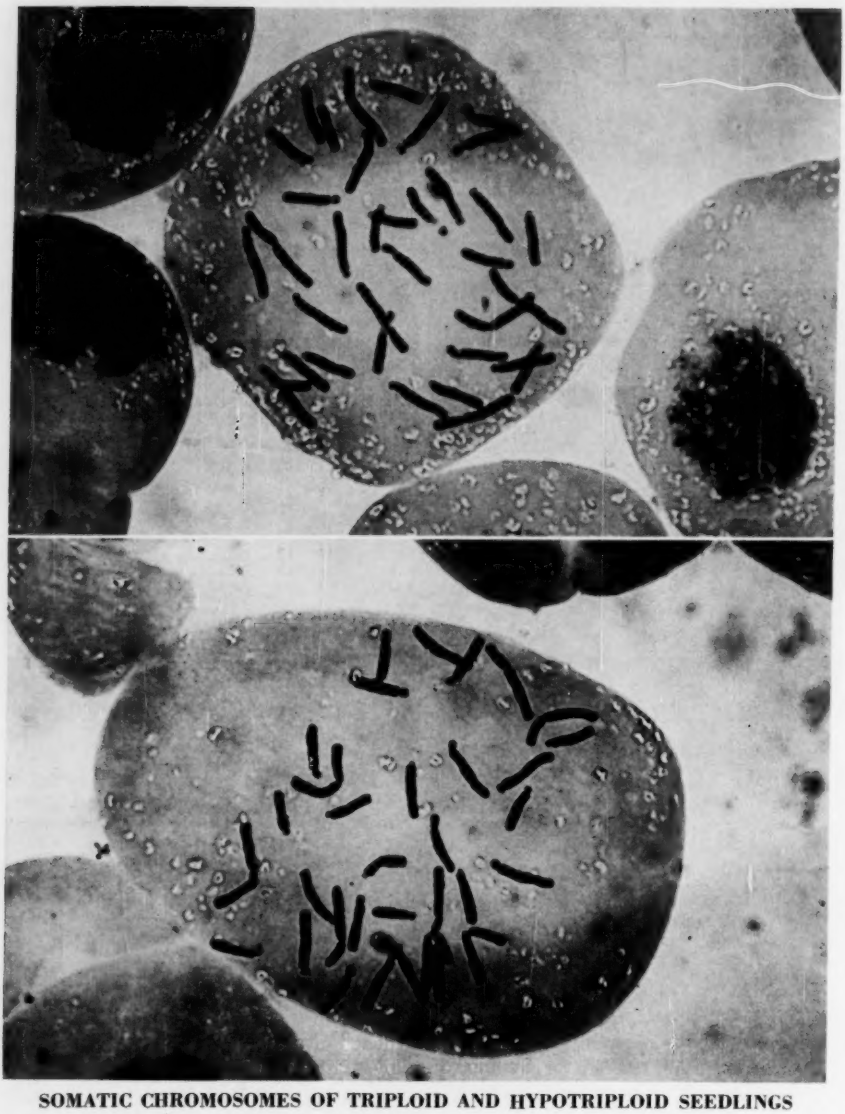
Two colchicine-induced tetraploids produced by Dr. S. L. Emsweller (U.S.D.A.) served as tetraploid stud plants. The first, LT38, is a colchicine-induced tetraploid of the USDA seedling #38 which, in turn, is a seedling from a cross between Croft and Creole. The second, LT73, is a colchicine-induced tetraploid derived from Creole.

In order to flower the plants in March, April and May when the temperatures in the greenhouse were fairly uniform and easier to control, the bulbs were precooled at 40 degrees F. for six weeks.

The determination of chromosome numbers was made as recommended by Stewart[18] with a few modifications. Hydrolysis and staining were accomplished simultaneously in a mixture of one drop of aceto-lacmoid and three drops of aceto-carmine in a watch glass over an alcohol lamp.

To promote fruit set, 1 percent naphthalene acetamide incorporated into a

*Research carried out at the University of Connecticut, Plant Science Department; Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission and the University of Florida, Botany Department. Gratitude is expressed to Dr. S. L. Emsweller for providing some of his experimental material. The author wishes to acknowledge the advice and encouragement extended during these investigations by Drs. G. A. L. Mehlquist and W. H. Camp and the criticisms offered by Dr. S. Shapiro in the preparation of this manuscript.



SOMATIC CHROMOSOMES OF TRIPLOID AND HYPOTRIPLOID SEEDLINGS

Figure 2

A—A cell from the root-tip of the triploid seedling, 54015-18, showing all 36 chromosomes. *B*—The somatic chromosomes from a root tip smear of the hypotriploid, 54015-29, with 34 chromosomes. Ca. ×512.

lanolin paste[7] was applied to the base of the ovary.

Results and Discussion

Pollinations

Before the breeding work was undertaken, a study of self- and cross-compatibilities among the diploids and among the tetraploids was made. This was desirable because the degree of self-compatibility governs the care needed in the emasculation of flowers and in the post-pollination treatment to guard against further accidental crossing.

Table I shows the compatibility relationship among the diploid clones. The data indicate that none of the diploids tested are sterile; that is, each clone is capable of producing seed or of acting as a pollinator. However, the data show that the diploid clones tested are self-incompatible.

The colchicine-induced tetraploid, LT73, was found to be self-incompatible. On the other hand, LT38, is self-compatible to a limited degree, having produced some 15 tetraploid seedlings thus far. Since one method of producing triploids is by making reciprocal crosses between tetraploids and diploids, attempts to produce triploid lilies were found to be limited by the number of tetraploid clones available. Therefore, intercrosses were made among LT73 and the selfed-population of LT38 in order to obtain a larger population of tetraploid seedlings. As a result, a population of about 136 polyploids was obtained.

Reciprocal crosses were thus possible between a large number of diploids and tetraploids. Using the diploids as the seed parent, some 569 flowers were pollinated resulting in 153 seedlings. In the reciprocal cross, some 284 tetraploid flowers were pollinated resulting in 40 seedlings.

Studies of pollen tube growth

Studies were made of the germination and growth of pollen tubes in the styles of Croft which were self- and cross-pollinated. It was found that upon self-pollination, the pollen tubes had traveled only about 22 mm. at the end of two days. Even after the sixth or seventh day these pollen tubes never reach the bottom of the style which is about 120 to 125 mm. in length. When cross-pollinated with USDA 44, the pollen tubes were found to be about 82 mm. in length after two days. On the fourth day after cross-pollination the pollen tubes were observed to be traversing the bottom 10 mm. of the style and to have completed its passage through the style by the fifth day. The observations made by Brierley *et al.*[1] in a preliminary study of pollen-tube growth in self-pollinated Creole also showed that the pollen germinates but penetrates only part of the way down the style. Humphrey[9] in a study on the behavior of pollen tubes in *Lilium regale* found that in self-sterile plants, the pollen tubes had progressed only 30 to 34 mm. or less than half the length of the style after 34 hours where the normal time of fertilization was 36 to 40 hours after pollination. Upon selfing *Lilium pardalinum*, which is self-sterile, Brock[2] found that the pollen tubes were normal in appearance and had reached the base of the style five days after pollination. The evidence thus indicates that there is some variation on the time of expression of the incompatibility reaction among the different species within the genus *Lilium*. Further data on the possible nature of this reaction is currently being sought.

TABLE I. Compatibility among the diploid clones

	Croft	Creole	Estate	USDA 44	LWQ Austr 38
Croft	—*	+†	+	+	+
Creole	+	—	...‡	+	+
Estate	+	+	—	+	+
USDA 44	+	+	+	—	+
LWQ Austr 38	+	+	+	+	—

*incompatible
†compatible
‡no data

Cytological analyses

The seedlings obtained from the successful crosses between diploids and tetraploids were analyzed cytologically for somatic chromosome number.

When diploids were used as seed parents, two triploids, one hypotriploid with 34 chromosomes, 132 diploids and 18 unclassifiable seedlings (nine died at a very early age, nine albinos died after the appearance of the cotyledon) were obtained. Unfortunately one of the triploids (54002) survived just long enough to produce one abnormal flower in the summer of 1954. The second triploid (54015-18) produced six normal buds of which three were allowed to flower on the first flowering shoot in the spring of 1955. The other three flower buds were removed for further cytological studies. Figure 1 illustrates the flowering spikes and individual flowers of this triploid (right) and of a sister diploid seedling, 54015-1 (left) during the 1956 growing season. The number of flowers and the internode lengths are not comparable since the diploid was not precooled whereas the triploid bulb was precooled at 40 degrees F. for six weeks. The flowers of the triploid are larger and heavier-textured than its sister diploid. This triploid grows very vigorously but its behavior in comparison to corresponding diploids will have to be deferred until a larger clonal population is established. Figure 2*A* shows a cell from the root-tip of the triploid with 36 chromosomes. The hypotriploid (54015-29) survived long enough to produce two abnormal flowers with intense anthocyanin coloration on the outside of the corolla on its first flowering shoot in the spring of 1955. Figure 2*B*

illustrates a cell from a root tip smear of this hypotriploid with 34 chromosomes. The occurrence of a large number of diploids from crosses using the diploid as a seed parent suggests that these seedlings may be apomicts. Further studies will be required to establish this point.

Using the tetraploid as the seed parent, five hypertetraploids (two with 50 and 3 with 49 chromosomes), 23 tetraploids, 2 hypotetraploids (47 chromosomes), one hypertriploid (37 chromosomes), 2 triploids, two hypotriploids (one with 35 and one with 33 chromosomes), and five unclassifiable seedlings (owing to death in the early seedling stage) were obtained. These seedlings have not attained flowering size. A detailed analysis of these seedlings will be reported in a subsequent publication.

It is evident that detailed studies of the mechanism of self- and cross-incompatibilities as well as the irregularities that occur during mega- and micro-sporogenesis are needed in order to establish the basis for the small number of triploids upon reciprocal crosses of diploids and tetraploids. In spite of the same problem of limited progeny, polyploidy has played an important role in the development of garden types of tulips and hyacinths which belong to the family Liliaceae and the narcissi in the family Amaryllidaceae. The tulips, hyacinths and narcissi have, however, developed from diploidy through triploidy and then to tetraploidy. The triploids in these cases have been presumed to arise from the fertilization of an unreduced egg by a haploid sperm[15,22].

Summary

The production of four triploids ($3n = 36$) from reciprocal crosses between diploid and tetraploid clones of the Easter lily, *Lilium longiflorum* is reported. One triploid (54015-18) has matured to produce flowers which are larger and heavier-textured than its sister diploid seedlings. Another triploid (54002) survived just long enough to produce one abnormal flower in the summer of 1954. Two others have not attained flowering size.

Eight-hundred and fifty-three crosses between diploids and tetraploids resulted in 193 seedlings. Using the diploids as seed parents, two triploids, one hypotriploid with 34 chromosomes, 132 diploids and 18 unclassifiable seedlings (nine died at a very early stage, 9 albinos died after the appearance of the cotyledon) were obtained. When the tetraploid was used as a seed parent, five hypertetraploids (two with 50 and three with 49 chromosomes), 23 tetraploids, two hypotetraploids (47 chromosomes), one hypertriploid (37 chromosomes) and five unclassifiable seedlings (owing to death in the early seedling stage) were obtained. These seedlings have not attained flowering size.

Data are presented which indicate that the diploid clones studied are cross-compatible but self-incompatible due to the cessation of pollen tube growth after traversing only one-tenth of the length of the style.

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THE SEX CHROMATIN OF SWINE INTERSEXES

GEORGE E. CANTWELL, E. F. JOHNSTON AND J. H. ZELLER*

IT has become necessary to determine the genetic sex of hermaphrodites in swine litters in order to establish the mode of inheritance of the condition. Barr[1] and his associates discovered a method by which a morphological distinction could be made between intermitotic nuclei of males and females of the cat. It was found that in the cells of genetic females there exists a special mass of chromatin, the sex chromatin, which is not present in high degree in the cells of genetic males. It is believed that this body is the heterochromatic portion of the XX sex chromosome complex. Moore *et al.*[3], used this method to establish the fact that the bovine freemartin was a genetic female.

The pigs in question form an extremely heterogeneous group anatomically, which includes true hermaphrodites, male pseudohermaphrodites, and those with gonadal dysgenesis. No animals exhibiting female pseudohermaphroditism have been recognized.

Methods and Materials

The experimental animals ranged in age from eight weeks to eight months and in weight from 15 to 292 pounds. The eight normal males and 10 females examined included purebred Yorkshires and Durocs, while the seven intersexed individuals were purebred Yorkshire or crossbreds between Durocs and Yorkshires. Gonads and the abnormal reproductive tracts of the intersexes were removed for histological study at the time of slaughter. Blocks from the following tissues and organs were taken for cytological examination: liver, heart, kidney, thyroid, pancreas, adrenal, cerebrum, dorsal root ganglia, sympathetic ganglia, cerebellum, and spinal cord. The specimens of non-nervous tissues were fixed in 10 percent formalin, nervous tissues were fixed in three different fluids; an acetic alcohol formalin water mixture, 95 per cent ETOH, and 10 percent formalin. All tissues were fixed for a period of 48 hours. Nervous tissues were fixed also at 24 and 72 hours. The schedule for dehydration and infiltration was similar to that used by Barr[2].

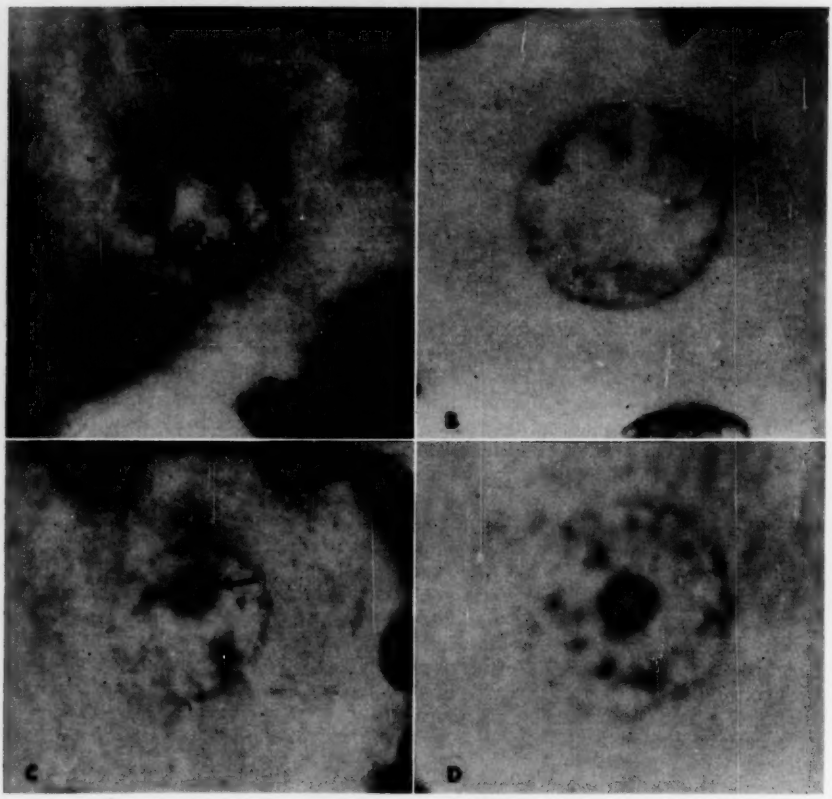
Sections of nervous tissue were cut at 10μ, non-nervous tissues were sectioned at 7μ. Both types of tissues were stained with hematoxylin and eosin, methyl green pryonin B, and by the Feulgen method. In the latter method, slices were hydrolized in N HCl for periods ranging from 2 to 30 minutes at 60°C. then placed in Schiff's reagent for 20 minutes. No counter stain was used in the Feulgen procedure.

Cytological studies were made with a standard binocular microscope under oil immersion. Measurements of the sex chromatin were made on 25 cells in each region of the nervous system using a filar micrometer. The incidence and position of the sex chromatin was recorded from 200 cells containing a nucleolus from each region of the nervous system.

Results and Discussion

The Feulgen method was the only method of the three that was used which revealed the sex chromatin and then only in nervous tissue. Moore[4] has also found this to be true. If the sex chromatin is present in non-nervous tissues of swine it is obscured by coarse heavily stainable chromatin clumps. Takahashi[6] demonstrated the sex chromatin in nerve cells of swine with cresyl violet but was unable to show it with the Feulgen method. We found the cerebrum and cerebellum to be less satisfactory than other nerve tissue because of the presence of other masses of chromatin. Both

*United States Department of Agriculture, Agricultural Research Service, Animal Husbandry Research Division, Beltsville, Maryland. The authors are indebted to Dr. Delbert T. Morgan, Jr., of the University of Maryland for taking the photographs.



NEURONS OF SWINE

Figure 3

A—Cell body from sympathetic ganglion of an intersex showing the sex chromatin body adjacent to nucleolar membrane (15 × 90 × 3). *B* and *C*—Dorsal root ganglia cells of normal females with sex chromatin next to nuclear membrane in *B* (15 × 90 × 2.5) and nucleolus in *C* (15 × 61 × 2.8). *D*-Neuron in dorsal root ganglion of male; sex chromatin is absent, (15 × 90 × 2).

male and female cells contained paranucleolar bodies that were Feulgen positive but these could be readily distinguished from the sex chromatin because of their much smaller size. The Feulgen positive body found in male cells was usually smaller than that observed in females; also the incidence of the body was considerably lower in male tissues.

With the Feulgen method the results varied due to time of hydrolysis and tissue. A recognition of the sex chromatin was almost impossible when any nervous tissues were hydrolized from 2 to 5 minutes. The most satisfactory time for hydrolizing cerebrum and cerebellum was 8 to 10 minutes and for spinal cord, dorsal root and sympathetic ganglia 15 to 20 minutes. Over hydrolysis (15 to 30 minutes) of the cerebrum and cerebellum led to errors in sexing due to over extraction of the DNA. No difference in results were noted due to the type of fixative used.

To determine the incidence and position of the sex chromatin, 200 cells each showing the nucleolus were examined from each region. The results are shown in Table I.

The average number showing the sex chromatin or in the case of males a mass that might be interpreted as sex chromatin was 95.2 percent in normal females, 93.5 percent in intersexes, and 7.2 percent in normal males.

Four intersexed animals resembled male pseudohermaphrodites according to the classification of Morris[5]. Each had a vulva, prostate gland, seminal vesicle, and an enlarged clitoris. The vagina was either small or absent. The uterus ended blindly at the seminal vesicle. There was no ovarian tissue present. Testicles with epididymides were located at the ends of the horns of the uterus. Histological examination of the testes revealed that spermatogenesis had not taken place, the seminiferous tubules were slightly fibrosed and many large Leydig cells were present. By the use of the sex chromatin test the genetic sex of these animals was concluded to be female.

One intersex appeared to be a case of gonadal dysgenesis. No gonads were found when it was slaughtered at about 200 pounds. The vagina extended from the vulva to the bladder with no cervix or uterus. The clitoris was not enlarged. The animal had a small nipple-like structure medial and just posterior to the navel. Examination of nervous tissues revealed it to be a genetic female, as indicated in Figure 3*A*.

Two animals were classed as true hermaphrodites, having both ovarian and testicular tissues. Neither had prostate glands, bulbourethral glands, nor seminal vesicles. Both had vulvae, vaginae, uteri and cervices. These animals were genetic females. No corpora lutea were present in the ovarian tissue nor had spermatogenesis begun in the testicular tissue.

The shape of the sex chromatin was usually spherical, or planoconvex due to flattening against the nucleolar or nuclear membranes, Figure 3*B* and *C*. The size of the sex chromatin (Table II) did not vary greatly between normal females and the intersexes.

The slight difference in shape of the sex chromatin in sympathetic ganglia cells probably was because here more of the chromatin bodies were flattened against the nuclear membrane.

Conclusions

It is possible to determine the genetic sex of males, females, and intersexes in swine by the use of the Feulgen method. Only nervous tissues were found suitable for cytological sexing. Non-nervous tissue does not reveal the distinguishing sex chromatin due to the presence of other coarse masses of chromatin. Of the nervous tissue examined the dorsal roots and sympathetic ganglia proved the most satisfactory. All of the intersexes examined were genetic females.

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TABLE II. Mean size of the sex chromatin (μ) in 25 neurons of each region

Region	Normal female	Intersexes
Dorsal root ganglia	0.8 × 1.1	0.8 × 1.1
Cerebellum	0.8 × 1.1	0.8 × 1.1
Spinal cord (ventral horn)	1.1 × 1.1	1.0 × 1.1
Sympathetic ganglia	0.7 × 1.2	0.7 × 1.2
Cortex	0.8 × 1.1	0.8 × 1.1
Average	0.84 × 1.12	0.82 × 1.12

TABLE I. Incidence (percent) and position of sex chromatin in various tissues of female, male and intersex

Tissue	Sex	Adjacent to nucleolus	Free in nucleoplasm	Adjacent to nuclear membrane	Total
Dorsal root ganglia	Gilts	72.0	11.0	11.0	94.0
	Intersexes	73.0	10.0	10.0	93.0
	Barrows	3.0	2.0	0.0	5.0
Cerebellum	Gilts	73.5	10.0	11.0	94.5
	Intersexes	70.0	10.5	14.5	95.0
	Barrows	5.5	1.0	0.5	7.0
Spinal cord (ventral horn)	Gilts	76.0	10.0	9.5	95.5
	Intersexes	74.0	9.0	11.0	94.0
	Barrows	3.5	1.0	1.0	5.5
Sympathetic ganglia	Gilts	56.0	9.0	32.0	97.0
	Intersexes	53.0	10.0	35.0	98.0
	Barrows	6.0	0.5	1.0	7.5
Cortex	Gilts	74.0	11.0	10.0	95.0
	Intersexes	71.0	9.0	9.0	89.0
	Barrows	7.0	3.0	1.0	11.0

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✦

ASYNAPSIS IN RED CLOVER

W. J. WHITTINGTON*

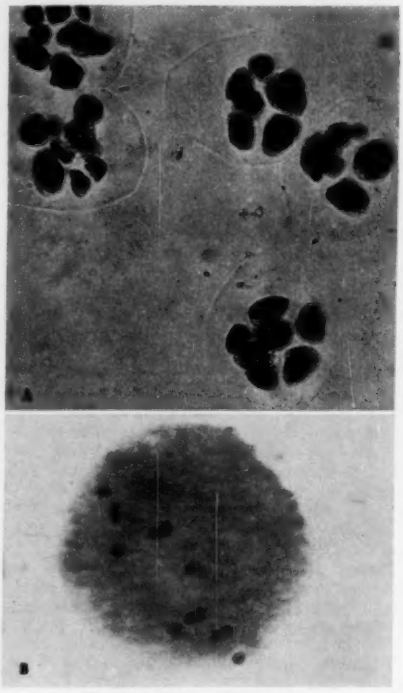
A PLANT of red clover, which was found to be sterile as either the male or female parent, was examined cytologically. Male sterility resulted from asynapsis with regular formation of 6-10 microcytes (Figure 4*A*). Since pollen grains from other plants germinated on the stigma and their tubes reached the ovary, it would appear that female sterility also results from an abnormal meiosis.

An examination of meiosis was made in the anthers from whole inflorescences fixed under reduced pressure in Carnoy's fluid. A trace of ferric acetate was added to the fixative and to the 70 percent alcohol used for storage. The chromosome complement was found to be 14 (Figure 4*B*), as is normal for *Trifolium pratense*. No chromosome pairing was observed, and the 14 condensed univalents could be observed in the cytoplasm. Chromosome separation took place to the poles, but the amount of movement and the number of chromosomes passing to each pole varied. It is likely that more than one spindle was formed, as was observed in maize[2], which resulted in several groups of chromosomes being established. The chromosomes were seen to be double, and their products separated, resulting in the distribution of single chromosomes or small groups of chromosomes within the cell. Wall formation around these chromosomes followed, but the pollen grains so formed soon degenerated.

The chromosomes of red clover are small and of similar size, and it was impossible—particularly in the absence of pairing—to determine whether a deficiency existed within any one chromosome.

These results are very similar to those reported for maize[2] and onion[3]. Asynapsis in both these plants led to the formation of microcytes, up to eight being found in the onion. There was, however, no variation of chromosome behavior in cells from different parts of the anther as in onion, and the regularity of asynapsis, in spite of environmental treatments, prevented a genetic analysis such as was possible in maize.

The study emphasizes the importance for continued development of a balanced set of chromosomes within the cell. Once a barrier to diffusion has been formed, such as the pollen grain wall, co-operation between abnormal nuclei ceases and the cells degenerate. Only if these barriers are incomplete, Barber[1], does development progress further.



ABNORMAL MEIOSIS

Figure 4

A—Pollen-mother-cells containing a variable number of developing pollen grains; × 400. *B*—Meiosis showing 14 univalents; × 1000.

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EVIDENCE OF NON-DISJUNCTION OF ABNORMAL CHROMOSOME 10

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THE cytological studies of Rhoades[1] revealed that the abnormal chromosome 10 in *Zea mays* behaves atypically at the metaphase and anaphase stages of meiosis by producing secondary sites of centromeric activity, usually in the long arms of the chromosomes. The number of chromosomes exhibiting such regions, which are called neo-centromeres, depends on the number of knobs. Rhoades[1] has also shown that the neo-centromeres move precociously to the telophase poles, and as a consequence cause the chromosomal arms bearing the neo-centromeres to become greatly attenuated, a condition which might lead to breakage. Moreover, Rhoades[1] has observed that occasionally both ends of a disjoining chromatid of a dyad exhibited neo-centromeres which were directed towards opposite poles rather than the same pole. This could result in either the production of fragmented chromosomes if the ends continued to move in opposite directions, or non-disjunction if one of the neo-centromeres ceased to function and the monad migrated to the same pole as its homologue. Evidence is presented below which supports the prediction that both breakage and non-disjunction occur in plants carrying either a homozygous or heterozygous abnormal chromosome 10.

Materials and Methods

The general scheme employed in detecting non-disjunction of the knobbed chromosome 10 (designated K) was based on the results of Hahn's experiment (unpublished) in which he measured the amount of crossing-over between the two closely linked components of the R gene complex. The procedure in his experiments was as follows: In cultures of R^g/r^r constitution the expected crossovers are R^r *and* r^g, but the r^0 class could also be accounted for by mutation of either of the parents. The R^r type, on the other hand, would not be expected to occur by mutation since dominant mutation is known to be rare at these loci. An alternative mechanism for the origin of an R^r individual from the R^g/r^r compound is by non-disjunction of the R^g and r^r chromosomes. If such be the case, the exceptional plant would appear phenotypically as a presumed R^r crossover but may be readily distinguished from a crossover by separate transmission of the R^g and r^r chromosomes in backcross progeny tests. Consequently, the occurrence of an individual of the R^r phenotype which is not due to crossing-over or to non-correspondence of embryo and endosperm genotype, represents the frequency of cases resulting from non-disjunction.

Three different R^g/r^r stocks were used as female parents in backcrosses to r^g/r^g, of which one was homozygous for the abnormal chromosome $10(K/K)$, another was heterozygous (K/k), and the third was homozygous for a normal knobless chromosome $10(k/k)$. The method followed in examining the backcross cultures was to plant the colored seeds in sand benches and to transplant all seedlings with plant color. These exceptional cases were grown to maturity and were examined cytologically for the chromosome 10 constitution.

Results

The results from tests of R^g/r^r cultures, with and without knob 10, are summarized in

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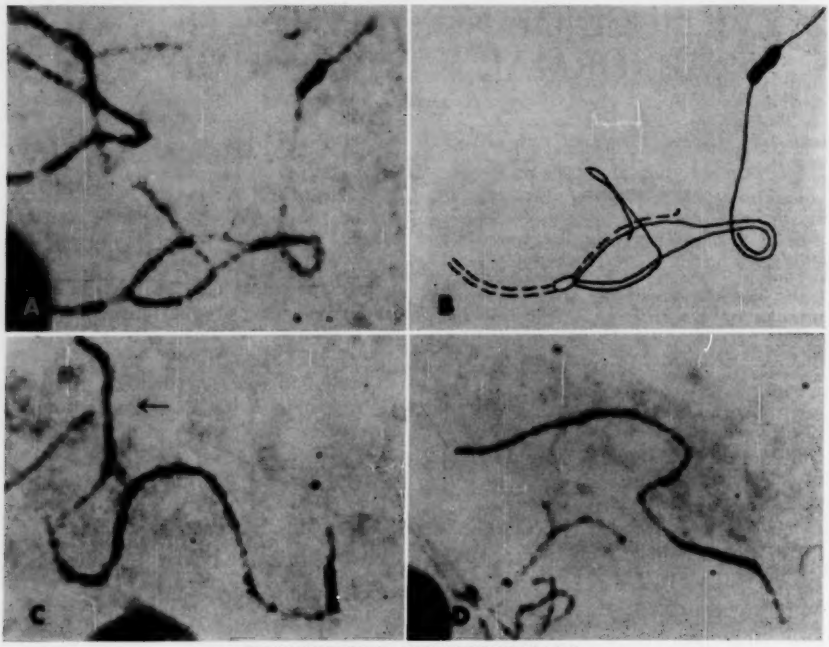


TRISOME FOR CHROMOSOME 10

Figure 5

A—Trisome for chromosome 10. *B*—Diagram of *A*. The short arm of chromosome 10 is drawn as broken lines; the long arm of chromosome 10 is represented as a solid line. The short arm of one homologue is paired non-homologously with the long arm. Also, the distal end of one long arm is paired with itself. *C*—Trisome for chromosome 10. The knobbed chromosome 10 is synapsed with one of the two knobless chromosomes. The other knobless chromosome (indicated by an arrow) is completely unpaired. *D*—Isochromosomes for the long arm of the abnormal 10. See Figure 6*A*.

Table I, along with the types of cytological aberrations observed. In progenies from the $R^s\ k/r^r\ k$ compound, without knob 10, no colored plants occurred among 24,319 colored seeds.

On the contrary, in cultures heterozygous for knob 10, totalling 11,424 seedlings, eight colored plants from colored seeds were found, and of these six were examined cytologically. In all six cases the plant concerned was found to be a trisome, and the knobbed and knobless chromosomes received from the heterozygous female parent were intact. Each of the six cases represents a separate event of meiotic non-disjunction, and the parent individuals occurred in three different cultures carrying knobbed chromosomes 10 of different stocks. Two of the six trisomes are illus-

TABLE I. Frequency of non-disjunction and translocations of the abnormal chromosome 10 in *Zea mays*

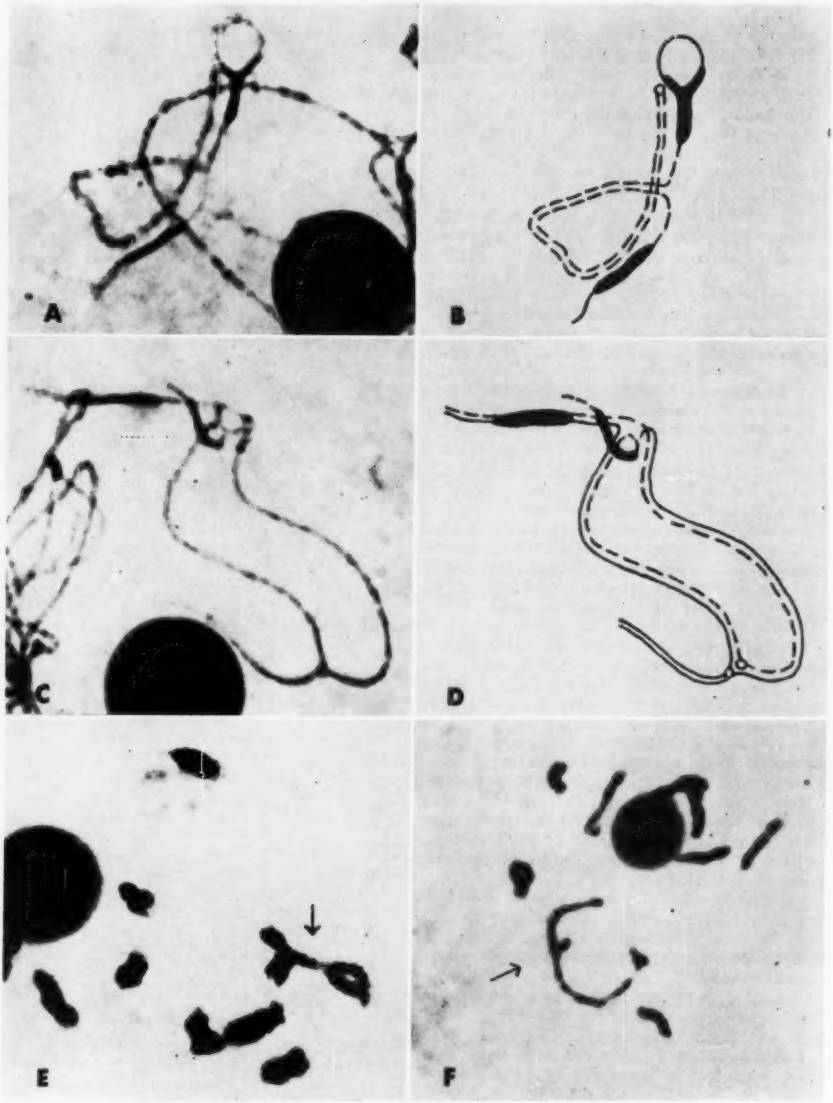
♀♀ R^g/r^r × ♂♂ r^g/r^g

Culture	Phenotype of colored seeds		Cytological results of colored plants		
	Colorless plants	Colored plants	Trisomes	Isochromosomes	Translocations
k/k	24,319*	0	0	0	0
K/k	11,424*	6 + 2†	6	0	0
K/K	806	8**	0	5	2

*Population counts obtained from Hahn's data (unpublished).

†Two colored plants not tested.

**One colored plant died before maturity but root tip examination revealed $2n + 1$ chromosomes.



ISOCHROMOSOMES OF ABNORMAL CHROMOSOME 10

Figure 6

A—Isochromosomes for the long arm of the abnormal 10. *B*—Diagram of *A*. The distal ends of the isochromosome are unpaired. *C*—Isochromosome synapsed with the normal knobbed and knobless chromosomes. See diagrammatic sketch. *D*—Diagram of *C*. The isochromosome is illustrated by broken lines; the normal knobbed and knobless chromosomes are represented by solid lines. The long arms of the knobbed and knobless chromosomes are paired with the isochromosome. *E* and *F*—Diakinesis cells with a possible duplicate-deficient type of translocation (indicated by an arrow).

trated at pachynema in Figure 5*A* and *C*. Figure 5*A* shows a typical trisomic configuration in which two of the short arms of chromosome 10 are paired homologously (short arm is indicated by broken lines in diagrammatic interpretation of Figure 5*A*), while the short arm of the third homologue is paired nonhomologously with part of the long arm (long arm is indicated by a solid line). Two of the long arms are synapsed homologously in the proximal region. In the distal region of the long arm, one of these chromosomes is paired with the long arm of the third homologue, while the other remains unsynapsed but folded back on itself in the distal region. Figure 5*C* shows a knobless chromosome 10 paired in its entire length with a knobbed chromosome. The third homologue of the trisome, indicated by an arrow, is completely unpaired with the disomic 10. The short arm of the univalent is synapsed with itself in the region of the short arm.

In cultures homozygous for the knob 10, eight seedlings with the R^r phenotype were found among 806 seedlings, of which five had isochromosomes, two had translocations, and one had $2n+1$ chromosomes in root tip examinations but failed to reach maturity. Since the frequency of exceptional plants was so high in the presence of homozygous knob 10, the parents of the backcross progenies (R^g K/R^g K and r^r K/r^r K) and the F_1 compound (R^g K/r^r K) were examined for the possibility of an aberration already present in the stock. Cytological observations of root tip preparations from each of the parents failed to reveal an extra chromosome 10: consequently, each of the isochromosomes must have originated independently in the backcross R^g K/r^r K to r^g k/r^g k. In view of the fact that translocations cannot be identified ordinarily in root tips, it cannot be ascertained at this time whether or not a translocation was already present in the stock.

Pachytene observations of each of the plants with an isochromosome for the long arm of the knobbed chromosome 10 had, in addition, an intact knobbed chromosome 10 from the female parent and a normal knobless chromosome 10 from the male parent. Figures 5*D* and 6*A* show isolated isochromosomes from two of the five cases, while Figure 6*C* illustrates still another case with an isochromosome paired with an intact knobbed and knobless chromosome 10. In Figure 5*D* the arms of the isochromosome are intimately paired with each other, whereas in Figure 6*A* the knobbed region is unpaired. It should be noted in this figure that part of one of the knobs, as well as the euchromatic strand distal to the knob, have split precociously. In Figure 6*C* the arms of the isochromosome (indicated by broken lines in Figure 6*A*) are synapsed with the long arms of the knobless and knobbed chromosomes (indicated by solid line); the short arms of the normal knobbed and knobless chromosomes are also paired.

Two of the eight plants with the R^r phenotype could be interpreted as carrying a duplicate deficient type of translocation, arising from adjacent-1 disjunction of a heterozygous translocation. These translocations were detected in separate backcross plants but were produced from the same stock. In the diakinesis stage both of these plants exhibited consistently eight bivalents and four associated chromosomes. It was also obvious from the diakinesis examination that the translocation configurations possessed two knobbed chromosomes 10 (Figure 6*E* and *F*). According to Rhoades[2], the abnormal chromosome 10 can be recognized at this stage of meiosis. Unfortunately, the cytological material was poor for extensive examination of the pachytene stage. The following limited observations were made at pachynema in both plants: (1) one intact abnormal chromosome 10 was present; (2) another chromosome of unknown identity (designated X) was seen with an attached knob-10, including the region of the dissimilar chromomeres of an abnormal chromosome 10; (3) these two chromosomes were associated in the distal regions of the long arm of chromosome 10; and (4) two other chromosomes were involved in this configuration of which one was a normal knobless chromosome 10. This means that the break point in abnormal chromosome 10 was proximal to the R complex so that the translocated chromosome received not only the knobbed region but also either the R^g or the r^r segment of the parent. It cannot be ascertained at the present time which of these segments was involved since further tests have not been made. In either case the intact knobbed chromosome 10 would have to possess R^g if the translocated chromosome included r^r or r^r if the interchanged chromosome possessed R^g since both the exceptional plants exhibited the R^r phenotype. Finally, disjunction in the heterozygous translocation was apparently of the adjacent-1 type which produced an egg with an intact abnormal chromosome 10(K) and the interchanged chromosome with the knob 10 region (X^{K10}). A gamete of this type would have a duplication for a portion of chromosome 10 but a deficiency for the region of the X chromosome which was translocated to the knobbed chromosome 10($K10^X$).

Discussion

The recovery of trisomes and isochromosomes from stocks carrying the abnormal chromosome 10, and the failure to find these aberrations in stocks with a normal knobless chromosome 10, strongly suggests that the mechanism giving rise to such abnormalities is related to the presence of abnormal 10. The simplest explanation might be that the neocentromeres are involved since the latter are known to occur only in stocks carrying the knobbed chromosome 10 (Rhoades[1]).

As mentioned before, non-disjunction could occur at the anaphase II stage, if the neo-centromeres of the disjoining monads were directed to the same pole. On this scheme it would be necessary to assume that crossing-over had occurred so that a dyad was produced with R^g and r^r, for the trisomes were of R^g/r^r constitution. Another possible explanation for the origin of the trisomes is that a chiasma failed to terminalize in time for the anaphase I separation, for the reason that the large knob acted as an obstacle to the chiasma. The resulting chromatid pair may then migrate to one pole.

The occurrence of the isochromosomes could also be attributed to neo-centric activity. However, in this case it would be necessary to assume that breakage occurred as a consequence of the mechanical stress imposed by the precocious movement of the neo-centromeres to the poles. If at anaphase I the chromatids break in the centromere region and the broken end of one long arm unites with the homologous long arm, an isochromosome would be formed. Another conceivable interpretation of the origin of isochromosomes is that misdivision of the centromere occurred. It is possible that this event could take place more frequently in stocks with the abnormal 10 than without, for the reason that the presence of neo-centromeres may cause an abnormal orientation of the centromere on the metaphase spindle plate. If a centromere should split vertically instead of horizontally, isochromosomes for the long and short arms of chromosome 10 would be produced.

A possible explanation for the origin of translocations is provided by observations made by Rhoades[1]. He found that in plants carrying the abnormal 10, chromosomes other than abnormal 10 exhibited neo-centromeres. Thus chromatid breakage resulting in reciprocal translocations between abnormal 10 and a non-homologous chromosome could arise as a consequence of neo-centric activity. However, it cannot be ascertained from the present cytological observations that these two cases of translocations represent independent events.

There is one remaining observation to be discussed. The large increase in aberrations in plants homozygous for abnormal 10, as contrasted with those heterozygous for abnormal 10, may also be ascribed to the neo-centromeres. Rhoades and Vilkomerson[3] have shown that the degree of neo-centric activity is much greater in plants homozygous for abnormal 10 than in plants heterozygous for abnormal 10. Consequently, it seems likely that chromatid breakage may occur at a higher frequency when two abnormal chromosomes 10 are present. If such be the case, then the frequency of aberrations should be high, as was found.

Summary

A cytological analysis was undertaken to determine the effects of the knobbed chromosome 10, commonly known as the abnormal 10, on the frequency of non-disjunction. The two closely linked genes of the *R* complex, which are located one unit from the knob, were used as genetic markers for detecting non-disjunction.

It was found that non-disjunction occurred only in plants carrying either a heterozygous or homozygous abnormal 10 and not in plants lacking this knobbed chromosome. This may be due to the fact that neo-centromeres exhibited only by the abnormal 10 cause the anaphase chromosomes to migrate irregularly to the poles and occasionally produce breakage of the chromosomes. Furthermore, a difference in frequency and type of non-disjunction was observed between plants heterozygous and homozygous for abnormal 10. The increased frequency of non-disjunction observed in the homozygote may be caused by an increased degree of neo-centric activity which is characteristic of homozygous abnormal 10. A satisfactory reason for the difference in the type of non-disjunction is not apparent at this time.

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PROGENIES FROM SESQUIDIPLOID F_1 HYBRIDS

Of *Lycopersicon esculentum* and *L. peruvianum*

ROBERT K. SOOST*

BOHN[1] reported the relative ease of obtaining sesquidiploid F_1 hybrids of *Lycopersicon esculentum* and *L. peruvianum* and suggested using these hybrids to obtain aneuploid lines having 26 chromosomes for breeding purposes. Because of the difficulty one encounters in crossing diploid *L. esculentum* with diploid *L. peruvianum*, the approach suggested by Bohn[1] was followed in the present study.

Materials and Methods

The seed parent for the initial cross was a tetraploid plant of *L. esculentum* (variety Pearson). A diploid plant of *L. peruvianum* (P. I. 129149) served as the pollen parent. Three flowers were pollinated on a single plant growing in a greenhouse during the winter. The fruits set readily and developed to normal size expected of the variety Pearson. Since only three fruits were available, no comparative measurements of fruit sizes were made. About 40 days after pollination one green fruit was harvested for examination of the developing seeds. Because seed development was more advanced than expected, no embryos were extracted at this time. When the remaining two fruits reached maturity, 16 embryos were extracted from them and grown on sterile nutrient agar as described by Smith[7].

All 16 embryos developed into extremely vigorous seedlings and were field-planted. The pedicels of all pollinated sesquidiploid flowers were treated with 0.01 percent *p*-chlorophenoxyacetic acid in lanolin[5]. Pollen viability of all plants was determined by examining pollen samples in aceto-carmine stain. Chromosome counts were made by using standard aceto-carmine squash technique on fixed material that had been premordanted with four percent iron alum according to the method adapted to tomatos by Dr. Marta S. Walters. Hematoxylin staining was used on all root-tip sections prepared for chromosome counts. All first-generation progeny of the sesquidiploids were grown in the greenhouse, and survivors were field-planted the following spring. Second-generation progenies were field-grown. Embryo culture was not used to secure the two generations from the sesquidiploids.

Sesquidiploids

Neither of the fruits from which the sesquidiploid embryos were extracted contained seeds that were completely normal. Even the largest seeds had greenish seed coats and rather soft endosperm. Bohn[1] found the same conditions in his crosses except when the fruits were allowed to ripen for several weeks after picking. Chromosome counts from pollen-mother-cell smears and root-tip sections demonstrated that all 16 plants had 36 chromosomes. Only 10 to 15 percent of the pollen from any of the plants was stainable. Repeated pollinations on each of the plants during the summer growing period failed to set fruit. Approximately 100 pollinations with diploid pollen and 50 with tetraploid pollen were made on each plant. Nine seedless fruits were set from 50 flowers pollinated with pollen from a triploid plant of *L. esculentum*.

Seventeen open-pollinated fruits containing 22 seeds were obtained from two of the plants. A third plant that died from curly-top disease (western yellow blight) produced 36 open-pollinated fruits. These 36 fruits contained only 17 seeds. The seeds in the open-pollinated fruits were normal in appearance

*Paper No. 1057, University of California Citrus Experiment Station, Riverside, California.

FLOWER AND LEAF TYPES

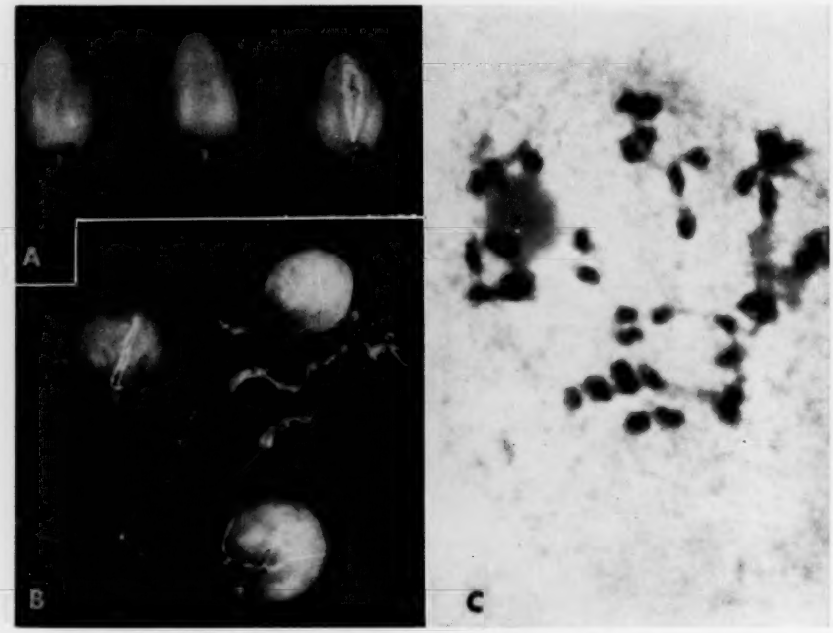
Figure 7

Plant characteristics of some first-generation progeny of sesquidiploids. *A*—From left to right: plants 55-2, 55-3 and 55-4; *B*—plants 56-1, 56-2 and 56-3. (See Table I for chromosome numbers.)

with brown seed coats and firm endosperm.

First-generation Progeny

The 17 seeds from the curly-top-infected plant produced five seedlings, three of which survived to maturity. The 22 seeds from the noninfected plants produced eight seedlings, all surviving to maturity. Flower and leaf types of six of



SESQUIDIPLOID FRUITS AND CHROMOSOMES

Figure 8

A—Fruits of plant 56-1. *B*—Plant 56-4. *C*—40 chromosomes of plant 105-7 at diakinsis.

these plants are shown in Figure 7. The characteristics of the plants resembled those of *L. peruvianum* more than those of *L. esculentum*. Since no seeds were produced by the sesquidiploids from the pollinations with *L. esculentum* pollen, it is assumed that the seeds set from open pollination were most likely the result of bee pollination with *L. peruvianum* pollen from the numerous accessions growing in the same planting. The chromosome numbers of these plants were indeed surprising. The chromosome number for four plants was 32, for three plants 33, and four had, respectively, the number 34, 31, 30, and 26. Pollen stainability ranged from zero to 60 percent. Chromosome number in itself did not determine the level of pollen stainability as evidenced by the fact that plants with identical chromosome numbers varied widely in percentage of stainable pollen (Table I).

In the greenhouse, plant 56-4 produced 12 parthenocarpic fruits and plant 56-1 produced 30 such seedless fruits. In the field, five plants, including 56-1, produced fruits from open pollination.

TABLE I. Chromosome numbers, pollen stainability, and fruit data of first-generation progeny of sesquidiploids

Plant no.	Chromosome no.	Percent stainable pollen	Total no. of fruits set	Total no. of seeds set	Average fruit wt. grams
55-1	32	60	0	0	–
55-2	33	50	22	8	1.5
55-3	32	5	0	0	–
55-4	32	5	13	13	1.6
55-5	31	1	0	0	–
55-6	30	0	0	0	–
55-7	32	30	51	95	2.4
55-8	33	1	0	0	–
56-1	34	30	40	2	3.3
56-2	33	5	28	73	4.2
56-3	26	5	0	0	–
56-4	–	60	0	0	–

SECOND-GENERATION SESQUIDIPLOID TOMATOES

Figure 9

Plant characteristics of some second-generation progeny of sesquidiploids. The chromosome numbers of the plants, from left to right, are as follows: *A*—24; *B*—25, 25; *C*—26; *D*—31, 28.

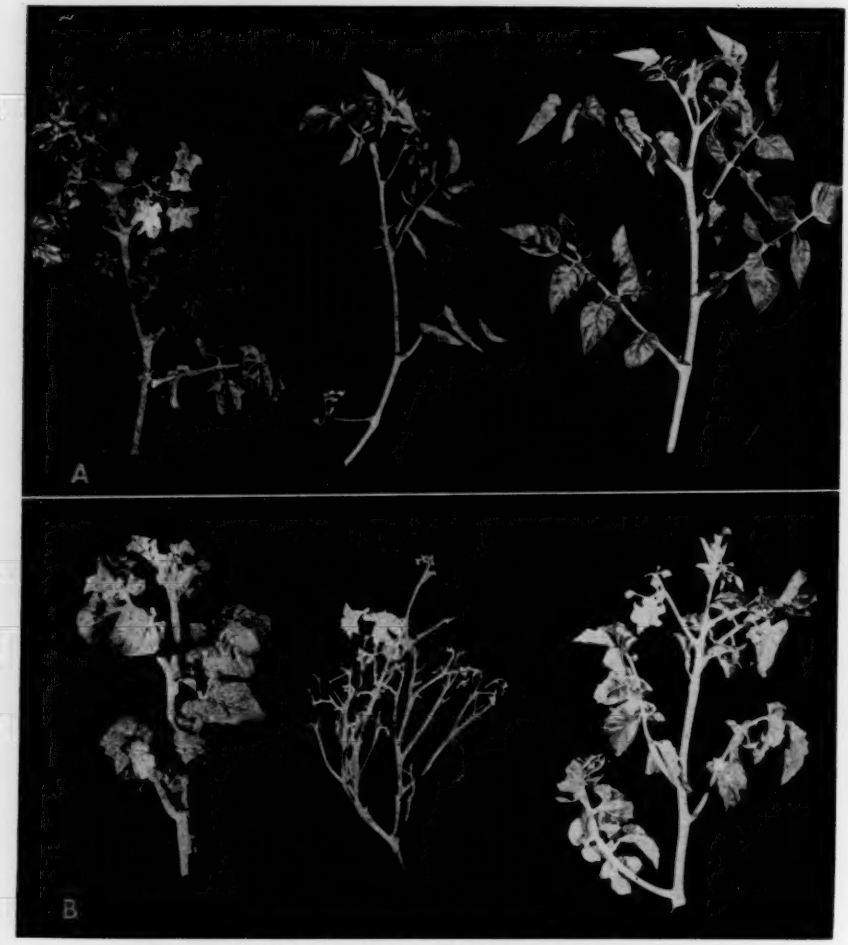
Plant 56-4 died before transplanting to the field. Table I shows the numbers of fruits and seeds set by these plants. As in the greenhouse, the fruits from 56-1 were mainly parthenocarpic, with 40 fruits producing only two seeds. The fruits of 56-1 and 56-4 are shown in Figure 8. Except for the fruits of 56-1 all fruits set were globose, and none of the fruits developed more than a slight buff color.

Second-generation Progeny

Four families were established from the seeds set from open pollination on first-generation plants. Eight seeds from plant 55-2 failed to germinate, but six plants were established from 55-4, nine plants from 55-7, one plant from 56-1, and 13 plants from 56-2. Many of these plants were extremely weak and died before chromosome counts were made. The chromosome numbers, which ranged

TABLE II. Distribution of chromosome numbers in second-generation progeny of sesquidiploids

Parent	Chromosome number of parent	Chromosome numbers of second-generation progeny							
		24	25	26	27	28	30	32	40
55-4	32	–	–	2	1	–	–	–	–
55-7	32	3	–	2	1	2	–	–	–
56-1	34	1	–	–	–	–	–	–	–
56-2	33	–	2	2	3	3	2	1	1



PLANT CHARACTERISTICS OF THE SESQUIDIPLOID

Figure 10

Plant characteristics of some second-generation progeny of sesquidiploids. The chromosome numbers of the plants, from left to right, are as follows: *A*—*27, 27, 27*; *B*—shows two 30 chromosome plants with the inflorescence of the left hand plant (105-13) in the center.

from 24 to 40, are indicated in Table II. Diakinesis in a pollen-mother-cell of the 40 chromosome plant is shown in Figure 8*C*. The chromosomes were usually paired in the three plants with the chromosome number of 24. Pollen stainability was always above 85 percent in two of them and above 60 percent in the third. Fourteen of the plants had three or more chromosomes in excess of the diploid number.

The wide variation in foliage characters exhibited by these plants is shown in Figures 9 and 10. Several plants were unable to produce mature flowers, and many flowered only at the end of the long summer season. Plant 105-13 (Figure 10*B*) produced rather compound inflore-

scences similar to that described by Rick and Smith[6] in crosses of *L. esculentum* with *L. peruvianum*. No attempt was made to pollinate these plants, and only plant 103-16 set seeded fruit from open pollination.

Discussion

Lesley[2] notes that when triploid tomato plants are crossed with diploid plants, random assortment of chromosomes in the triploids at meiosis should give 30-chromosome plants most frequently. However, plants recovered from such crosses never had more than 27 chromosomes, and most plants contained 25 chromosomes. Those plants having 26 or 27 chromosomes were also very much reduced in plant size. Rick and Barton[4] also note that the effect of two or three extra chromosomes is much greater than that of one extra and state that the upper limit of unbalance is three extra chromosomes. Only 0.09 percent of their plants produced from triploids had three extra chromosomes.

In contrast, the 11 plants from the sesquidiploids contained a maximum of 10 extra chromosomes. Whether the ability to carry extra chromosomes is due to the hybrid constitution of the plants or is due entirely to the *L. peruvianum* portion of the complement, there is certainly a marked difference from the behavior with an *L. esculentum* genotype. In this regard, it would be of interest to obtain aneuploids of *L. peruvianum*. The gene constitution of the *L. peruvianum* chromosomes may differ from that of the *L. lycopersicon* chromosomes, so that gene duplication is not as great in the hybrid material as it would be in *L. esculentum*. The fact that *L. peruvianum* is a self-incompatible species and therefore extremely variable should also add to the diversity of gene material involved.

The ability of these progenies to withstand increased numbers of chromosomes perhaps indicates a mechanism whereby new species with higher numbers of chromosomes could develop even though each of the parent species is able to tolerate only a few additional chromosomes. However, the presence of the self-sterility alleles[3] introduced from the *L. peruvianum* parent would make it difficult for self-sterile lines to be established.

Although the chromosome numbers obtained were surprising and of considerable interest, the failure to obtain an aneuploid line useful in breeding was disappointing. The high degree of pollen sterility of the sesquidiploids, coupled with the inability to effect crosses by using *L. esculentum* pollen, blocks progress toward an *L. esculentum* aneuploid type.

Summary

Aneuploids were established from sesquidiploids derived from crossing tetraploid *Lycopersicon esculentum* with diploid *L. peruvianum*. The inability to backcross to *L. esculentum* and the high degree of self- and cross-sterility prevented the establishment of *L. esculentum*-type aneuploid lines.

With the exception of one plant in which the chromosome number was 40, the chromosome numbers of the plants studied cytologically ranged from 26 to 34. The range of extra chromosomes carried by *Lycopersicon* aneuploids thus was extended beyond the previously known maximum of 27.

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HEREDITARY TREMOR IN DUCKS

S. DYRENDAHL*

SEVERAL hereditary abnormalities in the central nervous system of chickens have been studied and described[1-6]. Furthermore, nervous abnormalities of a hereditary nature have been recognized in other animal species. The present report deals with a hereditary tremor in ducks.

Description

A farmer in south-east Sweden bought seven eggs laid by crossbred ducks belonging to a neighbor who had kept ducks for a long time without any difficulties. These eggs were placed under broody hens. Seven strong and normal ducklings were obtained and when these were fullgrown, two ducks and a drake were retained for breeding. It is most probable that these three ducks were siblings. The ducks laid approximately 150 eggs of which the farmer retained 44 to be hatched under hens. The remaining hundred or so eggs were sold to several neighbors. Forty ducklings were obtained from 44 eggs kept by the farmer. Immediately after hatching it was observed that several ducklings in each clutch showed muscular fibrillations over the whole body, often to a pronounced degree. The head and neck were rapidly oscillated in an anterior-posterior direction. Affected ducklings had difficulty in maintaining balance. Of the 40 ducklings hatched, 10 showed signs of tremor. Most of the affected ducklings died within three days because of difficulties in taking up feed and a few were killed. Only one of the ducklings with tremor survived. This bird, a male, was made available for study in this department.

Of the 100 eggs which were sold, most of them hatched. Several of the neighbors who had bought eggs reported the occurrence of tremor among the ducklings obtained. Most of these ducks were killed immediately upon detection of the defect and it proved impossible to obtain information concerning the total number of ducklings with tremor. It is certain, however, that there were at least 18 affected ducklings of the approximately 90 which were hatched. A one-year-old female with tremor and an apparently healthy, full sister of this bird were submitted to the Royal Veterinary College by a neighbor to the original farmer.

Upon arrival here, the ducks were not quite one year old. The drake was in very poor condition and weighed only 1.3 kg. Tremor was quite pronounced and involved the entire body. Approximately 10 muscle contractions appeared per second and became more frequent when the bird was frightened or excited. The wings were partially involved in the tremor. Balance was only slightly disturbed but when the drake moved forwards, the tremor of the wings became more pronounced and approached flying movements. The tremor ceased when the bird lay at rest. This bird was unable to swim when tested in a bath. The muscle contractions became very strong in water and the drake had difficulty in keeping himself right side up. This bird seldom entered water voluntarily. Libido appeared to be strong.

The defective female displayed slower muscle tremors and although they were quite distinct, they were not so pronounced as in the case of the drake. Tremor mainly involved the head and neck while the wings were scarcely affected. Balance appeared to be undisturbed and the duck swam normally. According to the owner, the defective female had shown quite pronounced tremor when young but this had subsided somewhat at six months of age.

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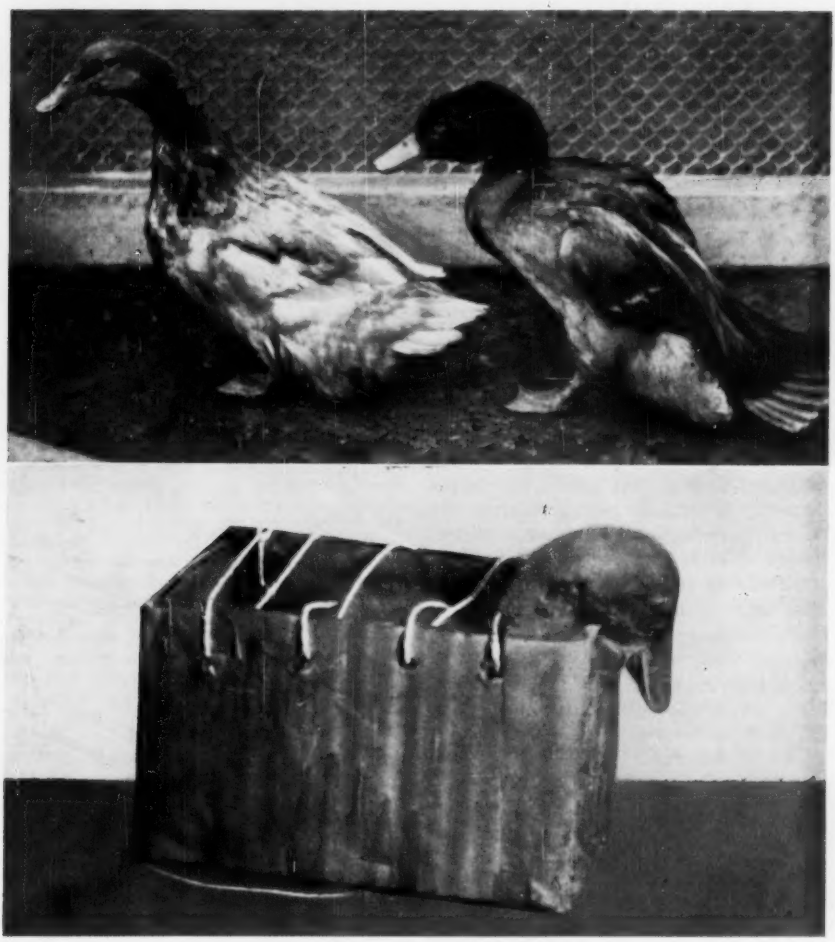

TREMBULOUS DUCKS

Figure 11

A—Shows two of the defective ducks which were submitted to the Veterinary College. The female (left) displayed slower muscle tremors. In the drake (right) tremor was quite pronounced and involved the entire body and wings. Balance was slightly disturbed and the animals were unable to swim. *B*—This duckling was placed in a series of paper boxes each larger than the previous one to accommodate the growing bird. This measure largely restrained the movements of the duckling and with the aid of force-feeding growth was possible.

After a short flushing period on a protein-rich ration, both ducks began to lay. The defective duck laid 30 eggs and the healthy duck 72. Of the 42 ducklings hatched from the eggs of the healthy duck, 19 were defective. Only five of the 30 eggs laid by the defective duck were fertile. Four living ducklings were hatched, all defective.

In many instances the defective ducklings were unable to leave the egg without assistance. Tremor was apparent immediately upon hatching. During the first days of life, balance was poor and

the ducklings often lay on their backs, unable to right themselves. Muscle tremor was quite obvious and involved the whole body. In all cases the tremor was so pronounced that the consumption of feed was markedly reduced or quite impossible. Tremor ceased when the ducklings lay down for sleep.

A few ducklings were injected with vitamin B_1 without any effect upon the tremor. Chloral hydrate given in the feed reduced the tremor for short periods only. Most of the affected ducklings died of starvation during the first week of life. One duckling, however, was kept alive for four months. This duckling had been placed in a series of paper boxes, each larger than the previous one to accommodate the growing bird. This measure largely restrained the movements of the duckling and with the aid of force feeding, growth was obtained. These efforts went unrewarded since the duckling finally hanged itself in the wire mesh of its cage.

Defective ducklings and the defective parents were autopsied. Although the central nervous system of many of these birds was sectioned at several levels and carefully examined, no abnormalities could be detected.

Discussion

Following the purchase of an unrelated drake by the farm where ducks with tremor first appeared, no more defective ducklings have been hatched. Both females at the Royal Veterinary College have been mated with several unrelated drakes. No cases of tremor have appeared among the ducklings resulting from these matings. There are good grounds, therefore, for assuming that the defect was caused by a recessive, autosomal gene. Affected ducks are recessive homozygotes (*aa*) which when mated with another cannot give rise to other than defective ducklings. The breeding experiments gave results which fully agree with this hypothesis. All of the four ducklings hatched were defective. Theoretically, the normal female could have been either a homozygous healthy individual (*AA*) or a heterozygous carrier of the gene (*Aa*). If this female was not a carrier of the gene, mating with the defective drake could not have given defective ducklings. Defective ducklings were in fact obtained. The female was obviously a heterozygous carrier of the gene for tremor. Mating the defective drake with the phenotypically normal duck should have given 50 percent healthy and 50 percent defective ducklings. This mating actually gave 54.8 percent healthy and 45.2 percent defective. The difference between the actual result and the calculated distribution is insignificant.

Summary

Several ducklings with congenital tremor were hatched on farms in south-east Sweden from eggs laid by inbred, crossbred ducks. Most ducklings died of inanition during the first days of life because of difficulties in consuming feed. Breeding experiments, however, were carried out at the Royal Veterinary College with a defective drake and a defective female. The tremor, which was quite pronounced and generally involved all the skeletal muscles, was evident immediately after the ducklings left the egg. Balance was generally poor and the ducklings often lay on their backs, unable to right themselves. No lesions of the central nervous system could be demonstrated histologically. Through breeding experiments it could be determined that the tremor was a recessive hereditary defect.

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TABLE I. Breeding results

		Eggs laid	Fertile eggs	Healthy ducklings	Defective ducklings	Healthy + defective
Defective ♂	× Defective ♀	30	5	0	4	4
	× Normal ♀	72	45	23 (11♂ + 12♀)	19	42

ABSENCE OF DIFFERENTIAL MORTALITY OF SEXES IN MICE

J. A. Weir, Harriette Haubenstock and S. L. Beck*

SEX ratio is just as much a property of a genetic isolate as are litter size or weaning weight. For example, from a listing of representative figures in the Handbook of Biological Data, page 519, we find that Jersey cattle produce 52 percent males at birth, Guernseys 42 percent; most breeds of sheep show an excess of females; man and the dog show an excess of males; the thoroughbred horse has exact equality of sexes. These figures do not lead to clearcut generalizations and a consideration of like data, as they relate to the effects of natural selection, led Darwin[1] to remark, p. 399, ". . . I now see that the whole problem is so intricate that it is safer to leave its solution for the future."

Our knowledge of the mechanism of sex determination might dispose us to expect the sex ratio at conception to be one male to one female, in which case we should try to isolate causes which act on the sex ratio after conception. But it must be admitted that this approach has not been fruitful. For example, in man, McKeown and Lowe[3] have shown that the various causes of abortion have different effects on the sex ratio and that the proportions of the different classes of abortions change from month to month during gestation. Other factors, such as non-randomness of museum specimens, the unreliability of classification of abortions occurring before the seventh month, and the difficulty of obtaining large numbers, add further complications.

It is our contention that there are no compelling reasons why we should attempt to fit the sex ratio at birth to a one-to-one sex ratio at conception. Certainly no assumptions of differential mortality are necessary to explain our results from mice[8]. For example, when mice of the high sex ratio strain, pHH, are crossed reciprocally with mice from the low strain, pHL, the sex ratio of the litter follows the male parent strain in each case. Also, artificial inseminations, made by using sperm from the ductus deferens, produce litters having the sex ratio characteristic of the sperm donor strain. These results seem to point to differential survival of X- and Y- bearing sperm rather than differential survival of XX or XY zygotes. But it is recognized that differential mortality may distort or modify the sex ratio; also with mice we can produce a large number of litters, each containing several individuals, and we can sex all of them.

We have accumulated data on the sex ratio at birth of live and stillborn mice, and of fetuses taken at the fourteenth and fifteenth days of gestation. To eliminate errors of classification we dissected all mice under a binocular sterioscopic dissecting microscope. Our experience has been that external morphology does not always provide a reliable basis for classification of sex at birth; occasional mice defy classification.

It turns out that the sex ratio of stillborn mice is no different than the sex ratio of living sibs and that the sex ratio at the fourteenth day of gestation is not significantly different from the sex ratio at birth or at weaning. Our data, like those of MacDowell and Lord[4] for mice and Tietze[7] for man, indicate that selective mortality is not necessarily a major cause for variations in secondary sex ratios among mammals.

Our outbred line of mice, which we

*Contribution from the Mammalian Genetics Center, Department of Zoology, The University of Kansas, Lawrence. This investigation was supported by research grants RG4878 and E-1412(C5) from the National Institutes of Health, United States Public Health Service.

have designated as T, is descended from animals obtained in 1949 from L. Butler, University of Toronto. He obtained the stocks from MacArthur[3], who started by crossing seven laboratory strains. We have maintained the line by systematic outbreeding designed to preserve maximum heterozygosis and there has been no detectable loss of variability in any of the several characters which are regularly classified. Over the years males and females have been produced in equal numbers in the T line but selection for blood-pH on two separate occasions, has given rise to lines that differ significantly from T in sex ratio.

The pHH and pHL strains, derived from T, are inbred, having been mated brother by sister for 18 generations by the time this study began. Neither strain is particularly difficult to maintain but production falls off rapidly in the pHH after the second litter. The blood-pH selection experiment which led to formation of the high sex ratio line, pHH, and low sex ratio line, pHL, has been previously described[9]. Work in progress, to be reported elsewhere, indicates that the association between blood-pH and sex ratio is complicated; the evidence does not support a simple cause and effect relationship.

Postnatal Mortality

Sexing by dissection, which is necessary for accurate determinations before or at birth, has the disadvantage that further observations are impossible. We did not obtain any new data on postnatal mortality in the course of the main study but did obtain information beforehand[8]. In 47 litters of the outbred T, of 338 mice classified at birth, 320 were living at weaning time. Of the 18 mice lost in the 30-day period, four were females and 14 males. Among 37 litters of pHH, pHL and F_1, the loss of 17 mice out of a total of 250 born included seven females and 10 males. These findings indicate that the male may be less likely than the female to reach weaning age but the difference is not significant for the pHH and pHL strains.

Sex Ratio at Birth of Live and Stillborn Mice

If the sexes are differentially susceptible to the vicissitudes of late intrauterine life, or to the hazards of parturition, we should expect to find a difference in sex ratio between live and stillborn mice. We have records for 475 litters of pHH and pHL in which all mice, live and stillborn, were sexed by dissection. There were 66 litters which had one or more stillbirths with a total of 283 living and 105 stillborn mice; and 409 litters with no stillbirths (2,815 mice).

It is well known that female mice frequently eat some of their offspring but we had no reason to believe that this contributed to the error to any appreciable degree. The mice were conceived and born in a reversed-light-cycle room and the observations generally were made soon after parturition. Even part-

TABLE I. Comparison of sex ratios of live and stillborn mice in litters including one or more stillbirths, (Expected values in parentheses)

Sire		Offspring			
Strain	Diet	Live mice		Stillborn mice	
		♀♀	♂♂	♀♀	♂♂
pHH	Acid	11	6	5	10
	Neutral	22	19	7	3
		33(31)	25(26)	12(14)	13(11)
pHL	Acid	64	37	12	10
	Base	31	25	13	7
	Neutral	36	32	24	12
		131(134)	94(91)	49(46)	29(32)

ly eaten mice, and those in the process of being eaten, could be sexed by our method, as a rule, since the mother usually proceeds in an anterior-posterior direction. The number of recorded stillbirths probably overestimates the true number as we had no way of knowing how many partly eaten mice were alive at birth; we recorded them all as stillbirths. Only four litters had stillborn mice which were not sexed and these litters are not included.

For litters containing stillbirths the data are summarized in Table I. The litters were sired by pHH and pHL males and within each strain there were three treatments, acid diet, alkaline diet, and neutral diet (the pHH, basic diet group, was represented by only one litter and has been omitted from Table I). Sex ratio is influenced by genotype of the sire and in some cases by diet of the sire; consequently the search for effects of differential survival has been made under a variety of conditions. This should serve to make the findings more widely applicable than would be the case if all mice were of like strain or on a single diet. The reasons for employing different diets are not relevant to the substance of this report and will be discussed elsewhere. No elaborate statistical treatment is necessary; the sex ratio among stillborn mice is not different from the sex ratio of living mice. The only additional point that seems worth making is to emphasize that live and stillborn mice were litter mates, a condition not met by routine laboratory records.

We do not wish to imply from our data that absence of differential mortality is a general phenomenon which applies to all strains of mice or under all laboratory conditions. Howard *et al.*[2] found that a drop in mean litter size between their preliminary and main series could be accounted for on the basis of increased prenatal mortality in females alone. On the other hand, Parkes[6] found evidence for selective prenatal elimination of males.

Since the sex ratio is the same for dead and living mice, without regard to strain or treatment, the live and stillborn groups listed in Table I may be combined for comparison with litters that contained no stillbirths. The results are shown in Table II. Again there were no significant deviations from expectation, calculated from marginal totals. Expressing the sex ratio in a more familiar form, percent males, the ratios were:

	No stillbirths	With stillbirths
pHH	47.3 ± 2.2	45.9 ± 7.4
pHL	41.0 ± 1.5	40.6 ± 3.7

Parenthetically, the above sex ratios show the influence of diet; the experi-

TABLE II. Litter sizes and sex ratios of litters containing no stillbirths compared with litters that had one or more stillbirths, (Expected values in parentheses)

Sire			Sex Ratio and Litter Size							
			Litters with no stillbirths				Litters with stillbirths*			
Strain	No.	Diet	No. litters	Mean litter size	♀♀	♂♂	No. litters	Mean litter size	♀♀	♂♂
pHH	2	Acid	66	7.7	242	205	8	4.0	16	16
	1	Base	16	5.6	55	34	1	2.0	1	1
	2	Neutral	65	6.2	198	205	9	5.7	29	22
	5		147	6.4	495(496)	444(443)	18	4.7	46(45)	39(40)
pHL	3	Acid	91	6.9	372	258	17	7.2	76	47
	4	Base	89	7.1	388	243	11	6.9	44	32
	3	Neutral	82	7.5	346	269	20	5.2	60	44
	10		262	7.2	1106(1107)	770(769)	48	6.3	180(179)	123(124)

*Litter sizes and sex ratios based on total mice, live and stillborn.

mental rations resulted in a lowered sex ratio in pHH mice.

Litters with no stillbirths and totals for litters with stillbirths now may be compared with respect to differences in litter size. It will be seen (from Table II) that litters with no stillbirths averaged 1.7 and 0.9 more mice than litters containing stillbirths for pHH and pHL respectively. This means that stillborn mice are not mere accidents of late gestation or parturition; more likely they represent the tail end of a gradual attrition. Also, since sex ratio is the same in depleted as in normal sized litters there is no reason to suspect differential prenatal loss. The next step should be to examine litters at the earliest stage of gestation which permits sex to be accurately detected by dissection.

Prenatal Sex Ratio and Mortality

Full-sib matings of pHH and pHL, and outcross T matings were made up according to standard practice. Females were checked twice daily for copulation plugs. At the fourteenth or fifteenth day of gestation each animal was anesthetized and the entire uterus removed. Initially, each fetus was removed into normal saline and the relative position of fetuses in the uterus was recorded. Each individual fetus, which was marked according to a system which involved amputation of the limbs, was transferred to Bouins picroformal for fixation. Later, the procedure was to fix the uterus whole, and to note the relative positions of the fetuses at the time of dissection. In addition to sexing, the order of living mice and of resorption sites were recorded, numbering from cervix to oviduct for each horn.

In horns having a large number of implantations the intervals between adjacent implantation sites usually were shorter than in horns having a smaller number of implantations. By the fourteenth day the sexes are easily distinguished grossly; the testis is more nearly round and more posterior than the ovary.

The sex ratios at last trimester, (see Table III), (pHH 55.9±3.9, T 52.9±3.1, pHL 45.0±3.4) do not differ from the previously published percentages of males for the three strains at 30 days of age (pHH 55.5±1.6, T 49.8±1.2, pHL 44.7±1.7). Also, litter sizes were similar; the third trimester followed by 30-day post natal means were: pHH 7.7 and 7.8, T 9.4 and 8.5, pHL 7.1 and 7.0. This striking agreement must not be construed as indicating that no further losses would have occurred among litters sexed at third trimester if they had been allowed to go to term and to weaning; apart from considerations already discussed in connection with stillbirths, there is direct evidence to the contrary. A small number of first litters, sibs of mice killed for second litter embryos, were saved and the newborn mice were sexed by dissection. Sex ratios with standard errors were: pHH 54.7±5.8, T 45.2±4.3, pHL 32.0±4.4. Litter

TABLE III. Sex ratios and resorptions in litters of pHH, T, and pHL mice collected at 14±1 day of gestation

Strain	No. of litters	♂♂ total living	% ♂♂ with standard errors	Resorptions No.	Resorptions %	Mean Litter Size At last trimester	Mean Litter Size At implantation
pHH	21	90/161	55.9±3.9	34	17.4	7.7	9.3
T	27	135/255	52.9±3.1	38	13.0	9.4	10.8
pHL	31	99/220	45.0±3.4	67	23.3	7.1	9.2

TABLE IV. Sex ratios and resorptions in litters above and below mean litter size (living mice)

	Above Mean Size						Below Mean Size					
Strain	No. of litters	♀♀	♂♂	R	% ♂♂	% Resorptions	No. of litters	♀♀	♂♂	R	% ♂♂	% Resorptions
pHH	13	50	68	14	57.6 ± 4.6	10.6	8	21	22	20	51.2 ± 7.7	31.7
T	17	90	99	24	52.4 ± 3.6	11.3	10	30	36	14	54.5 ± 6.2	17.5
pHL	15	76	55	27	42.0 ± 4.4	17.1	16	45	44	40	49.4 ± 5.3	31.0
Totals	45	216	212	65	49.5 ± 2.4	13.2	34	96	102	74	51.5 ± 5.0	27.2

sizes were: pHH—5.8, T—9.1, pHL—6.7. But even if the difference between third trimester and birth sex ratios for these particular pHL groups were all due to loss of males, the theoretical third trimester sex ratio for mice sexed at birth would be increased to only 35.8 percent males. The point need not be pursued further; there is no evidence for a relationship between sex ratio and litter size, except that in isolated instances litters smaller than usual have been associated with an unusually low sex ratio in pHH[8].

In the discussion of the preceding section, in which litters with no stillbirths and litters with stillbirths are compared, it has been pointed out that the incidence of stillbirths is correlated with earlier prenatal losses. Independent information from third trimester embryos has a direct bearing on this point (Table IV). The data, obtained by arranging litters of each strain in two groups, according to whether they fall above or below mean litter size, for living fetuses, taken at face value, indicate that (1) there is no obvious relationship between litter size and sex ratio and (2) the smaller litters have been depleted by post implantation losses. There were twice as many resorptions in the "small" as in the "large" litters. Except for the T strain the differences are significant. Because of the manner in which the data are arranged, only tentative deductions should be made from Table IV; we must return to the original records.

A comparison of groups arranged according to whether they fall above or below mean litter size, based on total implantation sites, is presented in Table V. For pHL mice, data from first and second litters had to be separated to prevent bias from disproportionate subclass numbers. The pHH group contained one litter that had two living females and 10 resorption sites; the sites were yellow and lacked the characteristic blood clot. But, in any case, there were no significant differences between "high" and "low" litter size groups with respect to resorptions (for example, for pHL, second litters, $\chi^2=1.25$, 1 degree of freedom). Crowding does not seem to be an important factor; it is largely a matter of the operation of unknown factors which determine whether a given embryo will survive or perish. Sex ratio is independent of total litter size. The high sex ratio of pHL second litters is unusual but an increase in proportion of males in second litters has been noted previously. It is of common, but not invariable, occurrence.

The findings presented in Tables IV and V, considered together, show that the number of mice at birth is a function of number of eggs implanted and of number of implantations surviving, and so far as can be determined these two factors are not correlated.

TABLE V. Sex ratios and resorptions in litters above and below mean litter size (total implantations)

	Above Mean Size							Below Mean Size						
Strain	No. of litters	♀♀	♂♂	R	% ♂♂	% Resorptions		No. of litters	♀♀	♂♂	R	% ♂♂	% Resorptions	
pHH	10	38	49	22	56.3 ± 5.3	20.2*		11	33	41	12	55.4 ± 5.8	14.0	
T	15	79	90	24	53.2 ± 3.8	12.4		12	41	45	14	52.3 ± 5.4	14.0	
pHL first litters	9	51	26	18	33.8 ± 5.7	18.9		8	22	19	8	46.3 ± 7.8	16.3	
pHL second litter	7	25	30	26	54.5 ± 6.7	32.1		7	23	24	15	51.1 ± 7.2	24.2	
	41							38						

*Includes one litter with 10 resorptions.

TABLE VI. Numbers of females, males, and resorptions in the left and in the right horns of the uterus of pHL, pHH, and T mice

	Left Horn					Right Horn				
Strain	♀♀	♂♂	R	% ♂♂	% Resorptions	♀♀	♂♂	R	% ♂♂	% Resorptions
pHL	33	42	15	41.6 ± 4.8	21.2	58	54	38	48.2 ± 4.8	25.3
pHH	57	66	10	55.3 ± 5.8	16.5	38	48	19	55.8 ± 5.4	18.1
T	63	45	29	53.6 ± 4.5	7.5	63	69	28	52.2 ± 4.4	17.5
Totals	153	153	54	50.0 ± 2.9	15.0	159	171	85	51.8 ± 2.8	19.7

Neither implantation number nor subsequent loss of embryos have any detectable effect on the sex ratio. Also, except for the already established strain difference in sex ratio, the pHH, T, and pHL mice have a similar type of reproductive pattern.

The method of recording fetuses and resorption sites, taking into account the relative position of each in the uterus, allowed for additional comparisons. The proportions of the sexes and frequency of resorptions in lateral and medial portions of the uterus and in "large" versus "small" horns, showed no consistent differences. Evidently the uterine environment is uniform throughout the length of the uterine horn. Crowding does not seem to be a factor of importance. On the other hand, a comparison of right and left horns of the uterus, although showing no significant differences in sex ratio, indicated a consistently higher resorption rate in the right than in the left horn. The numbers are small and the difference is significant only for the T strain. The total number of implantations is higher in right than in left horns (χ^2=3.90, P=.05).

Summary

Records for live and stillborn mice, and for fourteen and fifteen day fetuses have provided sex ratio data for three lines of mice, an outbred line, T, and two inbreds, pHH and pHL. Weaning sex ratios for the lines are pHH 55 percent ♂♂, T 50 percent ♂♂, pHL 45 percent ♂♂.

Among 475 litters of pHH and pHL in which all mice were sexed by dissection at birth, there were 66 litters with one or more stillbirths with a total of 283 living and 105 stillborn mice. There were 409 litters with no stillbirths (2,818 mice). There were no significant differences in sex ratio between live and stillborn mice, considering only litters containing stillbirths. Combining live and stillborn mice and comparing litter totals with the litters that contained no stillbirths the sex ratios, as percent males with standard errors were: pHH—no stillbirths—47.3±2.2, with stillbirths—45.9±7.4; pHL—no stillbirths—41.0±1.5, with stillbirths—40.6±3.7. [In this experiment the sex ratios were modified by use of special diets.]

Fetuses removed at the fourteenth and fifteenth day of gestation (mice on regular diet) had sex ratios, expressed as percent males with standard errors, as follows: pHH 55.9±3.9, T 52.9±3.1, pHL 45.0±3.4. These figures are almost identical with published means for 30 day postnatal sex ratios.

Comparison of litters grouped according to whether they fell above or below mean litter size, for living fetuses, showed no obvious relationship between litter size and sex ratio. There were twice as many resorptions in the "small" as in the "large" litters. A similar comparison, using total implantations, showed no significant differences in sex ratio or percent resorptions so crowding does not seem to contribute to prenatal loss. There were more embryos and a consistently higher resorption rate in the right than in the left uterine horn.

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SEX RATIO RELATED TO SPERM SOURCE IN MICE

J. A. WEIR*

TWO of our inbred lines of laboratory mice differ significantly from one another in sex ratio. The difference came about as an immediate correlated response to selection for the pH of the blood but was not at first recognized as an observation of significance. It was not until the breeding records up to and including the tenth inbred generation were analyzed that it was noted that the high blood-pH mice, pHH, had consistently produced an excess of males, whereas the low blood-pH line, pHL showed a consistent excess of females[6]. Except for the experiment of King on rats which, after six generations of selection, had produced a high male line (55 percent ♂ ♂) and a high female line (44 percent ♂ ♂)[4], there seems to be nothing even remotely comparable in laboratory mammals. Falconer's attempt to alter the sex ratio of mice by selection was unsuccessful even though the initial random bred stock was of such mixed origin that it should have contained much genetic variability[1]. The origin and subsequent history of our sex ratio lines, pHH and pHL, has been thoroughly documented, and the outbred foundation stock, T (50 percent ♂ ♂), has been perpetuated, so materials for investigating the causal mechanism of the difference in sex ratio are continuously on hand.

The rationale for the artificial insemination experiments presently to be described is based on evidence that we are dealing with differences in primary sex ratio, and not differential survival of zygotes. Results from reciprocal crosses, made over a period of three years, have firmly established that it is the sire that is responsible for the sex ratio of the litter[7]. This has focused attention squarely on the male so the problem becomes one of determining (a) the site of selective elimination of X or Y sperm and (b) the physiological basis for sperm selection. This paper is concerned only with the first aspect. The hypothesis is advanced that X or Y sperm are selectively eliminated, either in the epididymis or in the seminiferous tubules.

Methods and Results

The general plan was to inseminate females with sperm from the ductus deferens of males of one strain and to suspend the sperm in seminal plasma from males of the opposite strain. Since a natural mating is necessary to initiate in the female the neurohumeral chain of responses, which control implantation and lactation, a vasectomized male must be used. This male serves the dual function of providing seminal plasma and providing the mating stimulus. If the sex ratio of the litter follows the sperm donor it would seem that the selective effort is exerted during maturation or during storage in the epididymis; if the sex ratio is independent of sperm source or is influenced by seminal plasma, it would seem that for natural matings differential survival occurs after insemination.

Snell's technique for artificial insemination[5] was tried at first. Estrus was determined by vaginal smears; inseminations were made by inserting sperm directly into the uterine horns via the cervical canal, and then the female was placed with the appropriate vasectomized male. The technique for diagnosing estrus proved unreliable for pHH and pHL mice so it was decided to allow the mice themselves to determine the time of mating. For convenience the mice

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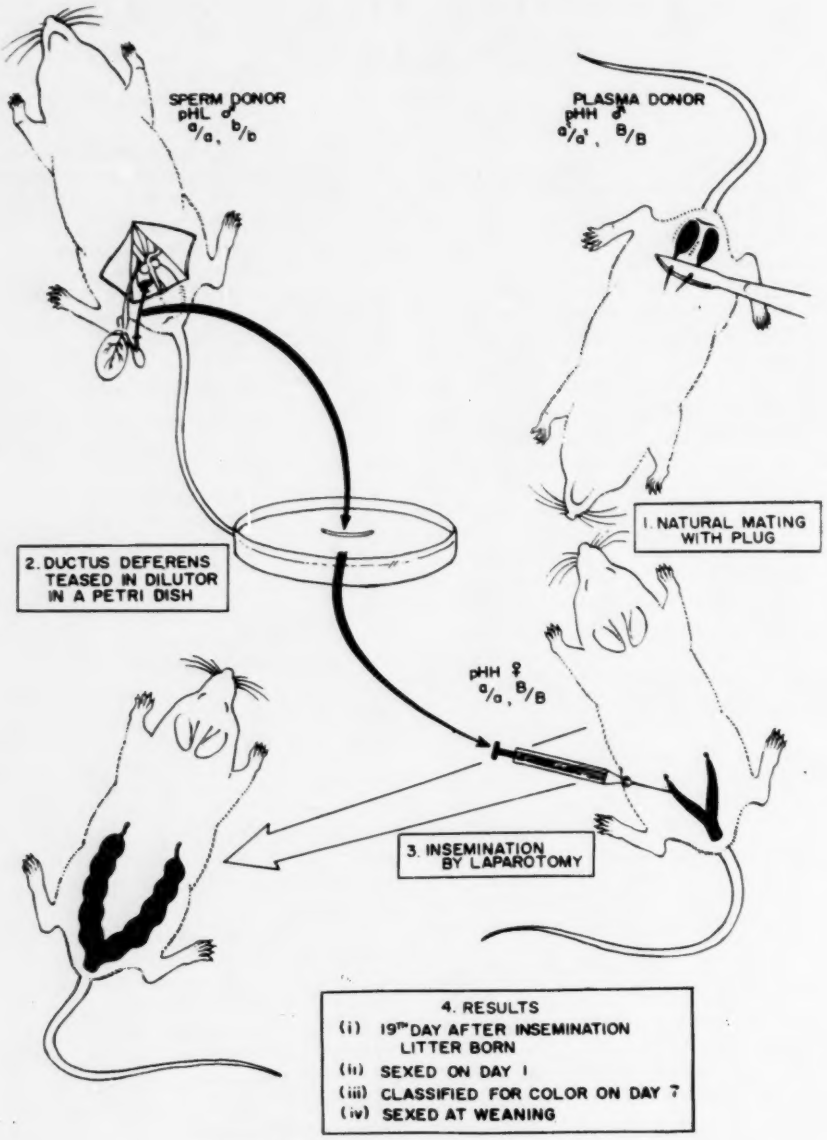

ARTIFICIAL INSEMINATION OF MICE

Figure 12

A test for influence of source of sperm on the sex ratio of mice, using the Kile technique for artificial insemination is shown diagrammatically.

were maintained in a room with reversed-light cycle with lights controlled to come on at 4 P.M. and go off at 7 A.M. Females were placed with the vasectomized males at 12 noon and examined for plugs at 1 P.M. They were examined again at intervals until 3 or 4 P.M. at which time unmated females were taken out of the mating cages. Females with plugs were inseminated by laparotomy. Before the experiment had gotten fully underway we learned of Kiles' technique[3] and have followed it with only minor modifications.

The sterile male was prepared by teasing apart the ductus deferens, taking care not to destroy the artery. One male used in the experiment became fertile as a result of regeneration of one duct so for subsequent cases the operation was modified; the duct and artery were sectioned. The blood supply to the testis is not affected by this procedure and mating behavior is unimpaired. The presence of one fertile male in the vasectomized group was at once detected because color markers were used to identify all litters.

The two inbred strains differ in color, and sublines within strains are also marked. All of the mice are non-agouti leaden but the pHH are black in contrast to the brown (chocolate) pHL. The four color genotypes are: pHH—*a/a, B/B, ln/ln* and a^t/a^t, *B/B, ln/ln;* pHL—*a/a, b/b, ln/ln* and a^t/a^t, *b/b, ln/ln*. By choosing appropriate color combinations for matings the paternity of the litter could be verified by the time growth of the baby fur was complete. One litter which could not be assigned by direct observation was identified by means of a testcross. These precautions proved fully warranted as it was from such evidence that the fertile plasma donor was located and the record of his progeny expunged from the experimental data.

Details of the procedure may be illustrated by reference to a typical example, as illustrated in Figure 12. 1. A black pHH female, placed with a black and tan, pHH, vasectomized male has recently mated as evidenced by a fresh viscous plug. 2. A brown male of the pHL strain is killed by cervical dislocation and opened down the midline, the ductus deferens is removed and placed in 0.65 percent saline (sperm remain viable for a longer period in 0.65 than in 0.90 percent saline). The duct is teased by forceps to remove the sperm, up to 0.25 ml. of the suspension is drawn into a 0.5 ml. tuberculin syringe. Sperm are examined under the microscope for motility and a 27 gauge needle is attached to the syringe. 3. The female is anesthetized under ether and opened down the midline. The uterine horns are lifted by a glass hook, the needle is inserted through the uterine wall, and the sperm suspension is injected directly into the

TABLE I. Sperm source and sex ratio of pHH and pHL mice

Results from artificial inseminations										
pHH sperm, pHL seminal plasma								pHL sperm, pHH seminal plasma							
Litters	♀♀	♂♂	♀♀ + ♂♂	% ♂♂	Standard Error			Litters	♀♀	♂♂	♀♀ + ♂♂	% ♂♂	Standard Error		
10	16	18	34	52.9	± 8.6			11	22	11	33	33.3	± 8.7		
Results from control natural matings															
pHH ♂♂								pHL ♂♂							
12	41	44	85	52.9	± 8.6			12	51	36	87	33.3	± 8.7		

uterus. The inner incision is closed by 000 surgical gut sutures, the outer incision by surgical silk. 4. If the insemination has been successful a litter arrives in 19 days and is sexed. As the young develop the sex is checked and by the seventh day they are classified for color. In this example, if the mice are all black it is evident that the black-and-tan vasectomized male did not contribute sperm.

By means of the Kile technique[3], 114 inseminations were attempted with 21 successes. The results are summarized in Table I. With each group of matings for artificial inseminations there were a few sib pairs for control natural matings; these were kept in the reversed light cycle room, placed with the males only during the early afternoon, and otherwise treated like the artificial insemination group. Unfortunately the females were not systematically checked for plugs, nor was there any attempt to record sterile matings, of which there were quite a few. As expected, the sex ratios of this control group were not significantly different from the sex ratios of contemporary mice in the main laboratory, although there was a slight overall reduction in number of males.

Sex ratios for control and stock natural matings were:

	Control group	*Stock group*
pHH	51.8 ± 5.4	55.6 ± 4.2
pHL	41.4 ± 5.4	43.7 ± 5.4

A two by two comparison from Table I, reading down first, shows that when pHH sperm were used for inseminations the sex ratio of the litter was not significantly different from the ratio obtained from contemporary pHH natural matings. Similarly the litters from pHL sperm were not significantly different from litters from natural matings of pHL. Reading across the table it will be seen that the differences between pHH and pHL are significant and of the same order regardless of whether sperm were introduced artifically in saline suspension, or were deposited by natural mating as part of the normal ejaculate. The X- and Y-bearing sperm are already disproportionate in frequency by the time the sperm reach the ductus deferens.

Discussion

If sex ratio can be modified by influences which act on sperm before ejaculation the problem of sex control becomes more sharply defined than before without our being much nearer to the ultimate solution. The sex ratio differences in our materials are genetic in nature; the determining causes are built into the mouse. Just as phenocopies of described mutants frequently come about as a result of non-specific treatments, so experimental modification of the sex ratio may result from treating males in various ways. We have in fact succeeded in altering the sex ratio of the litters by use of experimental diets, applied only to the male[2].

A disturbing feature in our artificial insemination experiments has been the small litter size; litters from artificial inseminations on the average were less than half the size of the control litters. Whatever the nature of the causes for such a drastic reduction in numbers, the sex ratio was unaffected. This finding may be construed as evidence that the sex ratio difference is truly a matter of frequency of sperm types and does not normally change after fertilization. This may seem to be an unwarranted generalization in the light of the great emphasis in the literature which has been placed on differential mortality. However, we do have some ancillary evidence. *First,* it has been our experience that unaccountable changes in sex ratio do occur in mice but that the effect is to reduce the proportion of one sex only; the difference between strains remains essentially unchanged. *Second,* we have found no evidence for differences in sex ratio between live and stillborn mice or between 14 and 15-day fetuses and full-term mice of pHH and pHL[8]. Admittedly, complete absence of information on mortality before the last trimester always leaves the sex ratio at conception an open question but for our material there is no necessity for making the assumption of differential mortality at any stage.

The presence of a fertile male among 30 vasectomized animals was at once discovered because of our use of genetic color markers. A curious uncontemplated experiment, as a result of mixing of two kinds of sperm, was performed and seems worth mentioning. The supposedly sterile male pHH 14320 leaden black, was mated to his full sister 14318 leaden black. Following this, sperm from the duct of pHL 14363 brown-and-tan were introduced directly into the uterus by laparotomy. Nine mice were born, a good litter, except that one mouse was small and seemed unlikely to survive. As the baby fur developed it became known that the small mouse was the only F_1

animal in the litter, the other eight were from the natural mating (legitimacy in this case is a matter of definition). At weaning the F_1 mouse, a female, was sleek of hair and quick of movement and weighed two grams more than the next largest litter mate, a half brother; heterosis had more than overcome the initial handicap.

Summary and Conclusions

Mice of the high sex ratio line, pHH, and low sex ratio line, pHL, were inseminated by the Kile technique. Natural matings to vasectomized males were used to illicit the progestational response of the uterus and females with fresh plugs were inseminated with sperm from the ductus deferens of freshly killed males. Sex ratios with standard errors for the different groups were: (1) pHH sperm—pHL seminal plasma 52.9±8.6, (2) pHL sperm—pHH seminal plasma 33.3±8.7, (3) pHH natural matings 52.9±8.6, (4) pHL natural matings 33.3±8.7. The results indicate that X- and Y-bearing sperm are already disproportionate by the time they reach the ductus deferens.

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◆

HEREDITARY DEFECTS IN DOMESTIC ANIMALS

SOME years ago an unnamed professor of animal husbandry, seeking to lighten the language requirements of students in an unnamed graduate school, declared that in his field there was nothing written in languages other than English that could justify the efforts of his students to acquire a reading knowledge of French or German. Perhaps so, but some day, some where, some specialist in animal husbandry, wandering afield from his punched cards and calculating machines, is going to see an abnormal calf or piglet and, perchance, to wonder whether its distinction is hereditary or not. This book* would probably tell him—if he could read it. There is no comparable up-to-date book in English that could do so.

All three authors are veterinarians. Before his recent retirement, Professor Koch was director of the Instituts für Tierzucht und Erbpathologie of the Free University of Berlin, and Dr. Schumann, an assiduous comber of scattered literature, was his assistant there. Professor Fischer is now director of the Institute of Animal Breeding in the University of Indonesia. Together they have compiled a useful survey of what is known about hereditary and congenital defects in cattle (100 pp.), horses (39 pp.), swine (64 pp.), sheep (42 pp.), goats (36 pp.) and poultry (116 pp.).

For each species the available information is arranged in appropriate sections. Some of these include all defects of one region of the body, or of one system, while others deal with some single condition not easily classified elsewhere. The general plan followed for each species can be illustrated by listing the main sections for cattle. These are as follows:

1. Abnormalities of the head and neck, including horns, ears, eyes, jaws, teeth and even De Groot's "smooth tongue," which is one of several pleiotropic expressions of a gene causing a peculiar hypochromic anemia.
2. Anomalies of hair and color ranging from too much, too little, and curly hair to albinism and tricoloration.
3. Abnormalities of the limbs.
4. Hypertrophy of muscles, the so-called "double-loin" condition.
5. Abnormalities of the vertebral column.
6. Hernia (umbilical).
7. Defects of the genital system including cryptorchidism, hypoplasia of gonads, testes that lie horizontally instead of vertically, defective Wolffian ducts, white-heifer disease, *impotentia coeundi*, and three kinds of abnormal spermatozoa.
8. Abnormalities of the udder and teats.
9. Nervous or hormonal disorders: spastic paresis, epilepsy, ataxia, nymphomania, dwarfism and gigantism.
10. Disease of the urinary system, one being porphyria, and the other albuminuria associated with nephritis.
11. Lethal genes, to the number of 31, with

*Erbpathologie der landwirtschaftlichen Haustiere. P. Koch, H. Fischer and H. Schumann. *XV* + 436 pp. Verlag Paul Parey, Berlin and Hamburg. Deutschmark 52. 1957.

their effects described in 35 pages with 20 illustrations and pedigrees.

12. Multiple births.

13. Check list of lethals, each briefly described.

The book becomes more useful when one realizes that the first attempt of the authors to classify congenital abnormalities in any species has been to put all those that are lethal in one group and those that are not lethal in another. These latter cases are then sorted out according to a plan more or less resembling that shown above for cattle. Accordingly, if a short-jawed calf dies, one looks for a similar condition under Section 11, where Numbers A_9, A_{14}, A_{18}, and A_{29} all have short jaws or none, but if the calf lives, its condition may be comparable to the brachygnathia described in Section 4.

Obviously such a system can lead to trouble. For example, the photosensitivity of lambs caused by an excess of phylloerythin is here classified with other lethal characters of sheep. Since such lambs are perfectly viable if kept out of strong sunlight, one wonders if the condition should not be listed simply as an abnormality of metabolism or of excretion. Similarly, it seems a bit complicated to discuss viable hypotrichosis of cattle on pages 10-12, but lethal hypotrichosis on pages 68-70. From these and many similar cases, it would seem to the reviewer that in future editions the authors might better scatter the lethals where these fit in with hide, hair or horn, and give for ready reference a simple check list of characters that are lethal or partially so.

For species other than cattle, the numbers of lethal characters listed are, for the horse: 10, in swine: 14, in sheep: 13, in the fowl: 34, in the turkey: 4, in the duck: 1, and for the pigeon: 3. None is given for the goat, a situation causing one to wonder whether the goat enjoys special dispensation or has merely been refractory to genetic analyses as to other things.

Most readers would want more evidence before admitting some of these cases to accredited status as simple recessive abnormalities. For example, in cattle the oft-reported mummified fetuses have not yet been proven to be caused by a single gene, and the evidence for cerebral hernia rests on only two cases. A sex-linked lethal gene may cause a deficiency of bull calves in some matings, but, since prenatal mortality falls more heavily on males than females anyway, this reviewer would like to see more evidence before admitting any such gene to the status of A_{21}. He has similar doubts about accepting for the fowl a supposed sex-linked lethal gene (E 7) and any simple recessive scoliosis.

It seems probable that most of the hereditary or congenital defects known (in the animals considered) are here described. For these reasons the book should be useful. The reviewer wishes that the authors had made some distinction between clearly genetic, adequately studied abnormalities and those assumed to be hereditary on the dubious ground of parental relationship. A congenital defect may be genetic in origin, or may not; for proof that it is a simple recessive condition, one would like to have controlled experimental matings, but these are often difficult to arrange in large domestic animals. Ratios in offspring from parents of known genotype are of value, but case reports of abnormalities seen in one or two related animals are of dubious value until supplemented by further information. Accordingly, the reader must not let illustrations of a headless lamb, a pig without hind legs, or a chicken with a crooked foot mislead him into thinking that everything pictured is inherited. On reading the text one finds that the evidence cited is often not as striking as the picture.

Including pedigrees, there are some 215 illustrations, many of them from original photographs by H. Fischer. Some of the pictures remind us of the difficulties inevitable in trying to get photographs of living animals that show a maximum of animal with a minimum of barnyard. Anyone who has ever tried to photograph a refractory month-old calf will acclaim the resourceful Mr. Krüger, who, in desperation, finally hoisted his hairless calf to the top of a post or box. There, immobilized, with its middle resting on the post and its legs dangling fore and aft, its plight was duly recorded for posterity, (Figure 52).

Some of the illustrations do not quite fit the context. The chicken shown in Fig. 164 is undoubtedly naked, but it has only an extreme degree of the late-feathering shown elsewhere, and *not* the sex-linked naked mutation discussed in three pages of context. Similarly, the chondrodystropic embryo in Fig. 208 should hardly be used to illustrate Asmundson's micromelia. Many of the best illustrations appeared first in the JOURNAL OF HEREDITY, but for these, as for all the rest, credit is given only to the original author—not to the original publication.

In deciding what is "Erbpathologie" and what is not, the authors have evidently adopted the guiding principle that sins of omission are worse than sins of commission. To this reviewer it seems odd that multiple births in cattle, sheep and goats should be considered pathological, and one doubts that Alexander Graham Bell ever thought of his multi-nippled sheep as belonging in that class.

Be that as it may, the very fact that the authors have attempted to include every abnormality known in the species considered will make the book valuable for reference. There is a glossary, a good index and an extensive bibliography that runs for 28 closely printed pages.

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CURVED LIMBS—A LETHAL IN DAIRY CATTLE

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A hitherto unreported lethal was seen in four Guernsey calves born in the Iowa State College herd. The abnormality seems certain to be hereditary. It may be a simple recessive although its mode of inheritance has not been established beyond all doubt. The facts are published at this time since they might help in genetic control and further evidence may not be forthcoming for some time.

The control of rare hereditary abnormalities is a conspicuous although minor part of the task of improving the general productivity of dairy cattle. Lerner[4] and Gilmore[2] give fairly recent reviews of lethals or near-lethal characters in dairy cattle. The one phenotypically most similar to the present case seems to be Ruzhevsky's report[6] of a semilethal condition in Friesian cattle resulting mainly in the fore limbs being bowed and ankylosis of fore and hind legs. Muscle contracture as reported by Tuff[7] involves posterior curvature of the limbs. Hutt[3] describes another case of muscle contracture as the same condition reported by Mohr[5]. Phenotypically, the abnormality of the Guernsey calves described below is different from those reported by the above authors[6,7,3], as far as can be judged from their descriptions, although all involve distortion of the limbs.

Description of the Lethal

Figure 13 shows the appearance of the affected calves. The deformity involved both muscular and skeletal growth. The rear legs were grossly deformed. The hocks were held close to the body and would scarcely flex forward. The distal part of the rear legs was strongly curved anteriorly and the cartilaginous tissue was obviously misshapen. The front pasterns were abnormally long and curved anteriorly. The thoracic cavity was constricted by the incurving sternum and the neck was slightly flexed dorsally.

Two of the four affected calves were stillborn and one other died during birth. The fourth lived for a few hours until dissected for a detailed anatomical description, but was incapable of sustained life. A detailed description† of No. 4450 is given below.

Skeletal abnormalities

1. The distal end of the femur was bent posteriorly, at right angles from the shaft, and was enlarged to twice normal diameter.
2. The tibia was bent at its middle, with the concave surface directed anteriorly.
3. All the costal cartilages were abnormal, being S-shaped instead of formed in a gradual semicircular curve.
4. The sternum was convexly curved (longitudinally and transversely) into the thorax. Normally the convexity of the sternum is away from the thoracic cavity, allowing more room for heart and lungs.
5. The shaft of the ilium was peripherally convex rather than concave.
6. The humerus, radius, and tibia were one-half to one-third normal diameter at their distal thirds.
7. The proximal phalanges were abnormally long.

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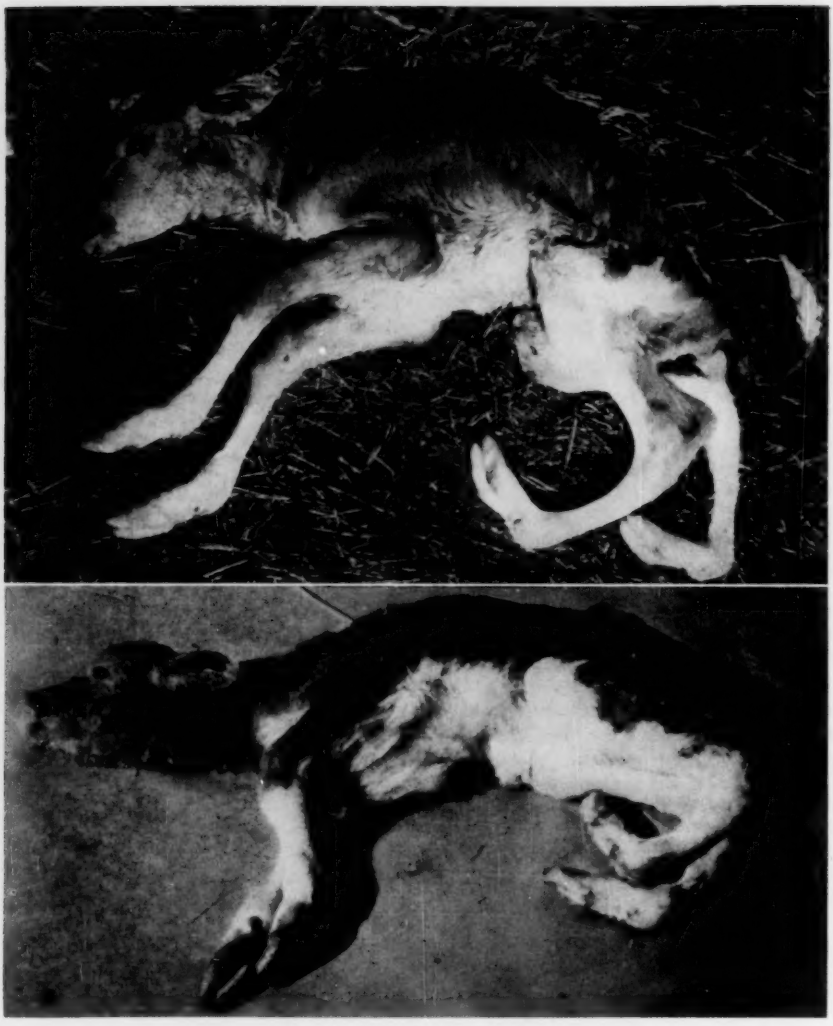
†Made by Dr. G. C. Christensen, Veterinary Anatomy, School of Veterinary Medicine, Iowa State College.



CURVED LIMBED CALVES

Figure 13

A and *B*—Shows deformed calf No. 4019 and No. 4450 respectively. (Photograph of *A* by Dr. L. D. McGilliard).

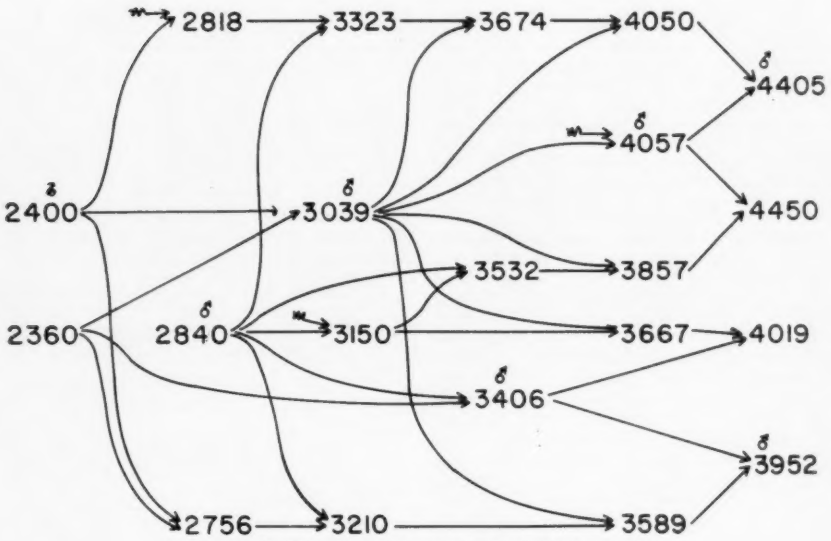


Figure 14

The four affected animals appear on the right. Incomplete arrows indicate unrelated ancestors.

Abnormalities of muscles and tendons

1. The brachialis was approximately one-half normal length and twice normal width. This acted to prevent extension of the elbow joint.
2. The extensor carpi radialis was short and thick.
3. The common digital extensor and both digital flexors of the fore limb were weak.
4. The biceps femoris, semitendinosus, and semimembranosus were short. They caused the femur to bend in utero and prevented normal extension of the stifle joint.
5. The tendon of the long digital extensor was too short, causing the anterior curve of the tibia and abnormal dorsal flexion of the hind fetlock joint.
6. The serratus ventralis thoracis was short and thick, causing the above mentioned distortions of the costal cartilages and sternum.
7. The complexus was shortened, preventing the animal from voluntarily flexing its neck. Also, the funicular part of the ligamentum nuchae was relatively short, helping to keep the head thrown back.

Herd History

The Iowa State College Guernsey Herd has been closed to outside breeding since 1949. The breeding plan was to linebreed as intensely as possible to cow 2360 who, considering both her own performance and that of her daughters, seemed much the best cow in the herd. Figure 14 shows the pedigrees of the four affected calves. The first of these was born in November, 1953, and the most recent one in February, 1957.

Approximately 120 cows of other breeds and old enough to calve are maintained in the same herd under similar management and nutrition. One abnormal calf of another breed was born during these 39 months but did not at all resemble these Guernsey calves in its abnormality.

Discussion

The mean gestation period for the four calves was 277 days, ranging from 269-282 days. This does not depart from the average of 283 days (Brakel *et al.*[1] and those they summarized from the literature) enough to indicate that this lethal condition shortened the gestation noticeably.

Bulls 4057 and 3406 each had affected progeny of which one was of either sex, thus, ruling out the possibility of sex-linked, sex-limited, or a sex-influenced type of inheritance.

That the character is inherited as a recessive lethal seems plausible. Cow 2360 alone could have introduced the lethal into the herd, since she is an ancestor of all four calves through both parents of each. The bulls 2400 and 2840 are also ancestors of all four calves but on both sides of only two affected calves, a different two in each case. If the character is inherited as a simple recessive, bull 3039 almost certainly was a carrier since his son from an unrelated cow sired two affected calves and 3039 himself is an ancestor of those two (No. 4405 and No. 4450) on both sides and of the other two on one side each. Bull 4057 sired 11 other normal calves in the herd. Cows 4050 and 3667 had only one calf each. The other two dams of affected individuals, No. 3857 and No. 3589, had three and five calves, respectively. These eight calves were by five different sires. Tentatively, we assume that this defect is a simple recessive although doubtless some more complicated genetic hypotheses would also be tenable as long as the evidence is this scanty.

Summary

Four grossly malformed Guernsey calves were either stillborn or born incapable of living for an extended period of time. Bone, muscle, and cartilage were deformed. The calves had rear legs curved forward, long fore and rear pasterns with the former also being curved forward, an incurving sternum and other less severe abnormalities. The character is clearly hereditary and presumably a simple recessive but the data were insufficient to exclude other more complicated hypotheses.

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ABNORMAL MYELIN AND LEUKOPENIA IN THE WABBLER-LETHAL MOUSE

A Suspected Case of Pleiotropism

MAURICE A. MUFSON AND ARNOLD STARR*

RECENT studies[3,7] attribute wabbler-lethal, a neuromuscular mutant in inbred mice, to a single recessive gene, which in the homozygous form (*wlwl*) produces a characteristic lethal infantile ataxia. The ataxic gait first becomes manifest at age 12 days, and progresses in severity until the animal dies; usually between 28 and 32 days of age. Non-wabbler sibs, normal (*WlWl*) and heterozygous (*Wlwl*) animals, do not exhibit neuromuscular symptoms and hence are outwardly indistinguishable. The wabbler-lethal gene is expressed in the homozygous form, the mating of heterozygous individuals yielding wabbler-lethals (*wlwl*) in approximately a 1:4 ratio.

Extensive investigations on the central nervous system of the wabbler-lethal[7,8] are being pursued in order to clarify the nature of the central nervous system myelin peculiarities and its relationship to human demyelinating diseases. In the present study a search for pleiotropic effects in other organ systems was undertaken in the hope that the elucidation of additional defects might eventually assist in the understanding of the mechanism of gene action in the wabbler-lethal mouse.

Materials and Methods

The progeny of mating pairs heterozygous for the wabbler-lethal gene (*Wlwl*) were used in these studies. The mating pairs of established genotype were obtained from M.M. Dickie of the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The animals were housed in small animal cages in temperature and humidity controlled rooms. Purina Lab Chow and drinking water were provided *ad libitum*. The progeny were kept with their mothers during the period of experimental observation.

Growth

Total body weight was used as an index of growth. At ages 7, 16, 20, 28 and 32 days each animal was weighed on a laboratory beam balance (accuracy $\pm$ 0.01 grams).

Peripheral Blood

At ages 7, 16, 20, 24, 28 and 32 days, peripheral blood samples were obtained from the progeny by the following method: The animals were warmed under a 50 watt light bulb for not more than three minutes. The tail was severed with a sharp razor, and the first drop of blood discarded. Two to three drops of blood were collected in calibrated red blood cell pipettes, and total cells counted using standard hematological methods. To ascertain the effect of repeated bleedings on the peripheral blood picture, tail blood samples were obtained from 32 day old non-wabbler siblings not previously bled.

Differential white blood cell counts (200 cells counted) were done on Wright-stained peripheral blood smears from progeny at 28 days of age.

Autopsies

Routine autopsies were periodically performed on wabbler-lethals and non-wabbler sibs. Tissue specimens for his-

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tological study were taken from heart, lung, skeletal muscle, liver, kidney, spleen, lymph node, bone marrow, adrenals, pituitary, thymus, and gonads. The tissues were fixed, embedded in paraffin, and stained with hematoxylin and eosin.

The data were evaluated statistically using analysis of variance for disproportionate subclass numbers and unequal means, thereby taking into account the variations between litters[10].

Observations

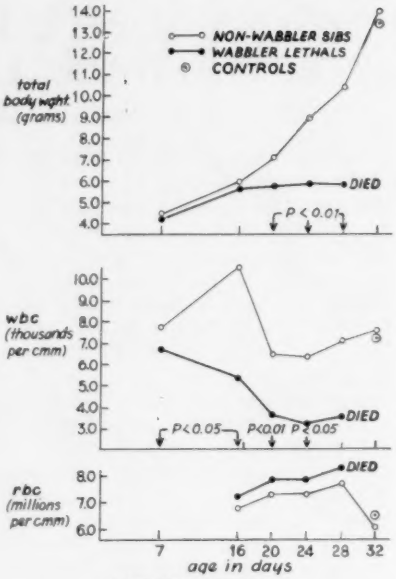
Growth

The total body weight in grams of wabbler-lethals and non-wabbler sibs at ages 7, 16, 20, 24, 28 and 32 days are presented in Table I. Wabbler-lethals gain weight at a normal rate up to 16 days of age, from 4.23 grams at seven days to 5.16 grams at 16 days. After 16 days of age and until death between 28 and 32 days body weight remains relatively constant, the growth curve describing essentially a plateau (Figure 15).

The non-wabbler sib gains approximately 0.6 grams per day during this period, from 4.41 grams at seven days to 13.90 grams at 32 days. Non-wabbler sib animals not used for bleeding studies (controls) weigh 13.38 grams at 32 days of age.

Peripheral Blood

The peripheral blood picture of the wabbler-lethal gives evidence of a moderate leukopenia as early as seven days of age, before growth abnormalities have become manifest, which progresses till their death usually between 28 and 32 days (Figure 16). Application of analysis of variance to these data demonstrates that at ages 7, 16, and 24 days the differences in the white blood cell count between wabbler-lethals and non-wabbler sibs are significant at the 0.05 level, and at age 20 days at the 0.01 level (Table I).



AGE VARIATION

Figure 15

The age variation of total body weight, white blood cells, and red blood cells of wabbler-lethal and non-wabbler sib mice.

Table II shows representative total white blood cell counts for seven litters at ages seven and 20 days.

At age 20 days the white blood cell count of the wabbler-lethal has a marked peak between 2000 and 4000 cells per cmm., while the counts of the non-wabbler sib are distributed symmetrically centering mainly at 5000 to 7000 cells per cmm. At age seven days this sharp

TABLE I. The age variations of total body weight, white blood cells and red blood cells of wabbler-lethal and non-wabbler sib mice

Age (Days)	Total body weight (grams) Wabbler-lethal	Total body weight (grams) Non-wabbler sib	White blood cells (per cmm.) Wabbler-lethal	White blood cells (per cmm.) Non-wabbler sib	Red blood cells (Millions per cmm.) Wabbler-lethal	Red blood cells (Millions per cmm.) Non-wabbler sib
7	4.23 (7)	4.41 (7)	6616 (7)*	7715 (7)	..	..
16	5.61 (8)	5.95 (8)	5306 (4)*	10513 (4)	7.21 (4)	6.80 (4)
20	5.71 (8)**	7.07 (8)	3608 (7)**	6494 (7)	7.83 (7)	7.33 (7)
24	5.79 (8)**	8.83 (8)	3219 (5)*	6305 (5)	7.84 (5)	7.27 (5)
28	5.76 (4)**	10.31 (4)	3497 (4)	7026 (4)	8.34 (4)	7.67 (4)
32		13.90 (3)	..	7548 (3)	..	6.06 (3)
32	Control	13.38 (3)		7215 (3)		6.46 (3)

The figures in parenthesis indicate the number of litters examined.

* P less than 0.05.

** P less than 0.01.

distinction is less marked, although the difference in mean total white blood cells between wabbler-lethals and non-wabbler sibs is statistically significant (Table I).

At age 28 days, differential counts on Wright-stained smears suggest that the leukopenia is referrable to a lymphopenia (Table III).

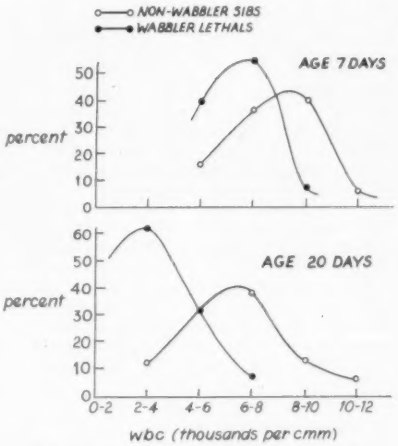
The total red cell counts of wabbler-lethals and non-wabbler sibs are shown in Table I. No significant differences in total numbers of red blood cells in the peripheral blood have been detected. Examination of peripheral blood smears did not reveal any red cell abnormalities.

Repeated bleedings apparently do not alter the total numbers of cellular elements in the peripheral blood as the determinations at 32 days on control animals, not previously bled, were similar to the non-wabbler sibs who were bled several times.

TABLE II. Total white blood cells (per cmm.) of wabbler-lethal and non-wabbler sib mice within litters, ages 7 and 20 days*

	Age 7 days		Age 20 days	
Litter	Wabbler-lethal	Non-wabbler sib	Wabbler-lethal	Non-wabbler sib
I	5661	5439	4440	5661
	7437	5661		
		9102		
		5106		
		8769		
II	7558	8880	3219	7569
	7104	10101		4329
	4773	8769		
		8214		
		7992		
III	6549	7770	2775	4440
		4884	2775	7770
		8436		
		10878		
IV	7992	6438	3330	3774
		9657	3996	9990
		9879		6549
		8214		
		7659		
		8103		
		7437		
V	9768	6438	5439	3108
	4773	7326	3330	9546
	7104	9102	5661	
		6660		
VI	6660	7770	6105	5550
		6216		6549
		9102		
		7548		
VII	5328	7104	2331	6660
	4440	5883	2997	4773
		9435	4329	6438
		4218		10212

*The frequency distributions of these data are presented in Figure 16.



FREQUENCY DISTRIBUTION OF WHITE CELLS

Figure 16

Frequency distribution curves of white blood cell counts for wabbler-lethal and non-wabbler sib mice at seven and 20 days of age.

Autopsies

Histological examination of hematoxylin and eosin preparations of other tissues did not reveal any degenerative or inflammatory changes. The bone marrow and lymphoid tissue were carefully studied and no alterations from the normal pattern were observed.

Discussion and Summary

The altered pattern of growth of wabbler-lethals, characterized by an early normal development during the first two weeks of life after which no further growth occurs, has also been observed in other mutant strains of mice, e.g. Jittery[2], Grey-lethal and Pituitary dwarfism[6]. One explanation to account for the initial normal growth of these strains may be that substances (hormones, metabolites) obtained in utero are essential for post-natal development. Assuming that the genetic defect in these mutant mice limits their ability to manufacture these factors, catabolism during these first few weeks would not be accompanied by an adequate replacement. In consequence, the course of growth is inhibited and the animals die prematurely. In Gray-lethal and Pituitary dwarfism dysfunctions of the parathyroid and pituitary, respectively, occur and the disordered growth in these animals has been attributed to these hormonal aberrations. Moreover, the administration of normal anterior pituitary extracts to the Pituitary dwarfism strain results in the restoration of normal growth. Wabbler-lethal and Jittery were originally investigated

because of their bizarre neuro-muscular behavior but the similarity of their growth pattern to that of Grey-lethal and Pituitary dwarfism, strains of mice without neurological abnormalities but with hormonal disturbances, is striking. Routine histological preparations (hematoxylin and eosin) failed to reveal any significant alterations in normal structure. It it anticipated, nevertheless, that the application of histochemical and physiological techniques may reveal hormonal or metabolic differences which would clarify the basis of the growth and development disturbances of the wabbler-lethal mouse.

The demonstration of a statistically significant leukopenia in the wabbler-lethal mouse, an animal with an established myelin derangement, attests to the diversity of expression of the wabbler (*wl*) gene. The leukopenia persists throughout the wabbler-lethal's life and is apparently referable to a decrease in the circulating mononuclear cells. At seven days of age, one week prior to the clinical identification of the wabbler-lethal, the individual white blood cell count cannot be utilized as a means of distinguishing wabbler-lethals from non-wabbler sibs, even though statistically significant differences were demonstrated for these two groups, since the frequency distribution curves of their white blood cell counts overlap widely. Later in the course of the diseases, however, the animal's phenotype can be correlated with its white blood cell count, an observation compatible with the progressive nature of the wabbler-lethal's disorder.

Although inherited leukemias have been demonstrated in a number of inbred lines of mice[4], the dyscrasia described in the present report is unusual in that the total white cell count is reduced and appears to be referable to a decrease in circulating lymphocytes.

Leukopenias have also been produced in mice by chronic starvation[9], "viral-like" agents[1, 11], and dysfunctions of the hypothalamic-pituitary axis[5]. The presence of a statistically significant leukopenia at seven days of age, at a period of normal growth, mitigates against inanition as an important factor in the etiology of the wabbler-lethal's low white blood cell count. Further, the wabber-lethal's myelin sheath peculiarities and concomitant leukopenia may be correlated with the homozygous expression of a recessive gene and it appears unlikely that an infectious agent is responsible for these traits. The possibility remains that the genetic constitution of these animals may render them more susceptible to certain environmental factors which might be initiating elements in their disease. The role of the hypothalamus in regulating many body functions either directly, through neural means, or indirectly, through the pituitary hormones, is well established. Myelin peculiarities can be demonstrated in the hypothalamic portions of the medial forebrain bundle and hypothalamic-tegmental tract in wabbler-lethals as early as eighteen days of age[8]. The Marchi method, used in these studies, specifically visualizes abnormal myelin and one might anticipate that other hypothalamic alterations could be demonstrated at a still earlier age if more sensitive histochemical techniques were utilized. It may be that the observed hypothalamic alterations will prove to be essential to the disturbances of growth and peripheral blood in the wabbler-lethal.

The lack of statistically significant alterations in the red blood cell counts suggest that the leukopenia is not secondary to an abnormality of the hematopoietic system as a whole, but rather to a relatively specific cellular defect. In view of this it also appears likely that the leukopenia is a primary disorder, characteristic of the pathological genetics of the wabbler-lethal mouse.

The findings suggest that the wabbler-gene is pleiotropic in action, exhibiting effects on such diverse targets as the myelin sheaths and the white blood cells. The mechanism of action of the wabbler-lethal gene in producing these manifold effects on two apparently unrelated tissues remains to be elucidated.

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TABLE III. Differential white blood cell count in wabbler-lethals and non-wabbler sibs: Age 28 days

	Lymphocytes	Neutrophils	Immature granulocytes	Monocytes	Eosinophils	WBC count Average
Wabbler-lethals						
Percent	38.5	59.5	1.0	0.5	0.5	–
Total	1540	2380	40	20	20	4000
Non-wabbler sibs						
Percent	69.0	28.5	0.5	1.0	1.0	–
Total	6210	2565	45	90	90	9000

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PLANT BREEDING AND CYTOGENETICS

IN spite of a wealth of material, textbooks and reference works that seek to combine the findings of cytogenetics with the principles of plant breeding are virtually non-existent. Professor Elliott has set out to fill this gap in our bookshelves, and on the whole has succeeded admirably.* He is well equipped by training and experience for this task, having spent his graduate days at the University of California and Iowa State College. For a number of years Professor Elliott taught plant breeding to both undergraduate and graduate students at Washington State College where he was a member of the Agronomy Department before transferring to the Farm Crops Department of Michigan State University in 1957.

A rough idea of the content and coverage of the book can be gained by a glance at the titles of the 13 chapters. The first chapter discusses "The nature of crop varieties", then follow five chapters primarily concerned with cytogenetics. Their titles are: "The chromosome mechanism in heredity" (Chap. 2); "The significance of recombination in plant breeding" (Chap. 3); "The role of mutations in plant breeding" (Chap. 4); "Polyploidy and plant breeding" (Chap. 5); "Chromosome substitution and aneuploid analyses" (Chap. 6). Chapters 7 to 12 deal almost exclusively with plant breeding as evident from the titles that follow: "The function of introduction in plant breeding" (Chap. 7); "Hybridization and selection of self-fertilized crops" (Chap. 8); "The improvement of cross-fertilized crop plants by hybridization" (Chap. 9); "Breeding for disease resistance" (Chap. 10); "Testing plant materials before release as varieties" (Chap. 11); "Handling plant materials after release as varieties" (Chap. 12).

There is a final chapter on "Organization for plant improvement" (Chap. 13). This chapter outlines the functions of those government bodies set up to administer funds allocated to publicly supported institutions for plant improvement both in this country and abroad.

Professor Elliott is frank to admit that solutions to all of our plant breeding problems are not included within the covers of this book, since in his words it is neither a "manual nor a cookbook." However, he has culled the essentials from a great deal of scattered material, and organized it into an integrated picture of plant breeding and cytogenetics. An unusual feature is the judicious choice of examples from a wide range of crop species to illustrate plant breeding and cytogenetic phenomena, rather than depending exclusively upon one group of crops (*e.g.* cereal crops). As a result, the reader learns something about crops as widely separated phylogenetically as pineapples and guayule.

Chapter 4 ("The role of mutations in plant breeding") written in collaboration with R. A. Nilan is an excellent up-to-date account of this increasingly important subject. The authors are concerned principally with mutation-inducing radiations and chemical mutagens. Their discussion will be of much value to the practical plant breeder because it is stripped of the excess technical verbiage that makes so many papers in radiation genetics tedious and difficult reading.

To cope with the alarmingly large array of diseases, insect pests and problems of adaptability to specific environments, the plant breeder should have at hand a representative cross section of the genotypic variability within the species, and of species that may be expected to exchange genes with those that are to be improved. In a chapter entitled, "The function of introduction in plant breeding," Professor Elliott has given serious attention to this problem. After outlining the main centers of origin of our important crop plants, the remainder of the discussion is occupied with the function and organization of those agencies in this country

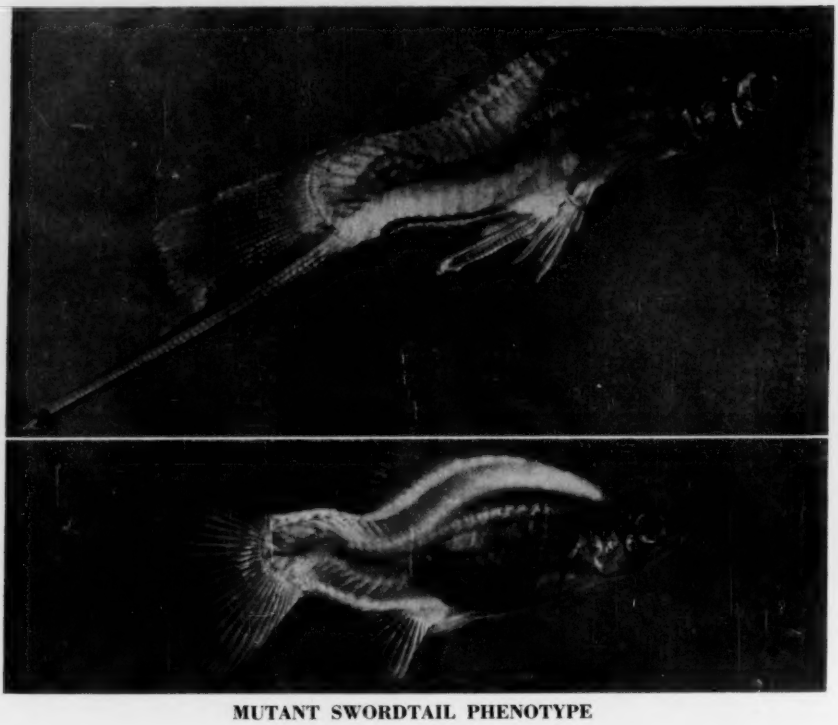
(*Continued on Page 242*)

**Plant Breeding and Cytogenetics.* Fred C. Elliott, 1-395, f 1-1 to 9-13, McGraw-Hill Publications, New York, 1958. $8.50.

SPINAL CURVATURE

A Mutation in the Swordtail, *Xiphophorus*

HAROLD L. ROSENTHAL, PAUL R. MYERS AND MARTHA K. BRUNINGS*



MUTANT SWORDTAIL PHENOTYPE

Figure 17

A and *B* shows the male and female mutant phenotype of spinal curvature in *Xiphophorus* sp.

DEFORMITIES of the spinal column appear to be common abnormalities among fishes and other vertebrates and in fishes, specifically, skeletal deformities appearing as "crooked spines" have been described in paleontological specimens[2]. Recent studies have shown that skeletal deformities in fishes may be hereditary. Aida[1] reported on the inheritance of two vertebral mutations in *Oryzias*, an egg laying cyprinodont, which he described as "fused" and "wavy". A similar mutation in *Lebistes* has been independently report-

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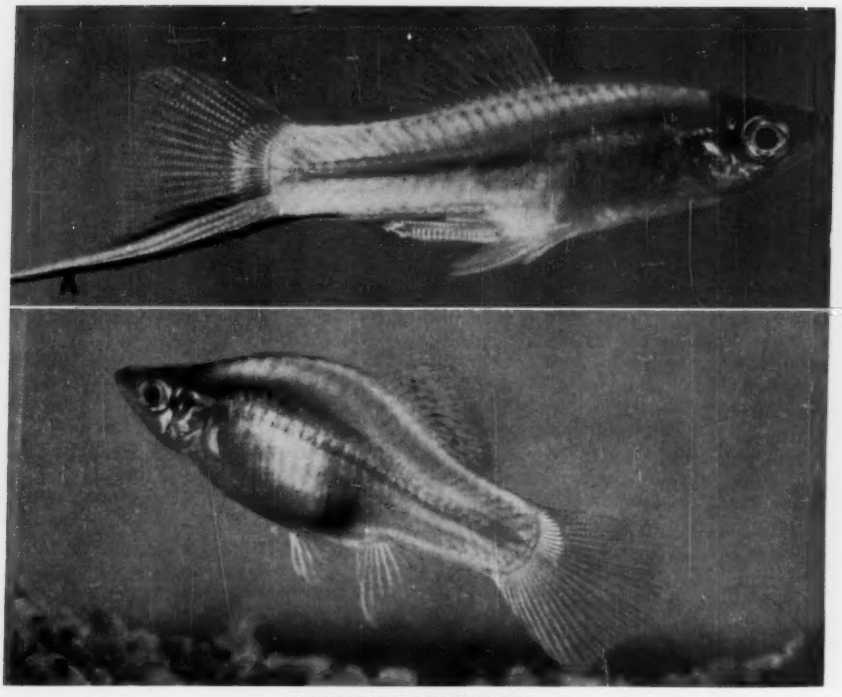


NORMAL PHENOTYPE

Figure 18

A and *B* shows the male and female normal phenotype in *Xiphophorus* sp.

ed by at least three other investigators[4, 5, 7] in which the phenotype was described as "hunchback" or "lordosis". The three reports for *Lebistes* appear to be, in all probability, studies of the same mutation. In all of the investigations with *Oryzias* and *Lebistes,* the inherited abnormality behaves as an autosomal, single-factor Mendelian character recessive to normal and independent of all known color genes and sex.

However, all skeletal deformities in fishes may not be due to genetic mutation. Thus, Foster *et al.*[3] induced skeletal abnormalities in rainbow trout with x-radiation prior to spawning and obtained a high percentage of fry with various kinds of skeletal deformities. However, the fry were not viable and genetic studies could not be made. More recently, Plugfelder[6] pinealectomized embryo guppies and found that the fish developed vertebral abnormalities as they aged. It appears to us that studies of skeletal deformities in naturally occurring mutations may yield information concerning bone growth and mineral metabolism not obtainable by other means.

The present report describes the spontaneous appearance of inherited spinal curvature in Mexican swordtail hybrids (*Xiphophorus*) which is similar to but not identical with that previously described in *Lebistes*.

The mutant swordtail phenotype is shown in Figure 17, and may be compared with the normal condition shown

in Figure 18. The vertebal column of normal and mutant swordtails and guppies are shown in Figure 19 for comparative purposes and it is apparent that the abnormality is phenotypically similar in both genera of fishes. The recessive allele is designated *Sc* (spine curved), the normal being *Sn* (spine normal) in accord with the previous designation for guppies[7].

Experimental Details

In 1952, a curved gravid female hybrid swordtail was obtained from a commercial tropical fish hatchery which gave birth to 20 young. The brood consisted of seven curved males, five curved females, seven straight males and one straight female. A working hypothesis designated the original female parent as a homozygous recessive (*ScSc*). The mixed brood must consist, therefore, of the homozygous recessive (*ScSc*) and the heterozygous normal (*SnSc*). The unknown male parent must also have been heterozygous normal (*SnSc*).

In order to test this hypothesis, the young were isolated at one month of age in separate aquaria until sex could be determined. This procedure assured non-fertilized females, a necessary condition for breeding studies with the live bearing cyprinodonts. The virgin females from this initial brood were crossed with the males in separate aquaria. As the females became noticeably gravid they were isolated to individual aquaria heavily planted with *Ceraptopterus* to prevent cannibalism of the new born young. The conditions for maintaining the fish through maturity and breeding were similar to those previously described in detail for *Lebistes*[7]. Successive broods of known genotype from individual fish were raised in separate aquaria and used in other breeding experiments. All broods were examined daily and the curved young were isolated. Normal appearing young were maintained until sexual maturity before discarding in order to check the possibility of late development of spinal curvature. Spines from mutant fishes raised in the laboratory and normal fish obtained from commercial sources were obtained as previously described[9]. In all cases, the eviscerated carcass weight was determined in order to avoid the abnormal body weights of gravid female fish. The spines were dissected free of adhering tissue (Figure 19) and air dried. The dry bone was defatted with ether, dried to constant weight at 100° C., ashed at 550° C., dissolved in 1 normal hydrochloric acid and diluted to 10 ml. Aliquot portions were analyzed for calcium and phosphorus as previously described[8].

Results and Discussion

The results of the breeding experiments are shown in Table II. Analysis of the data by means of the chi-square distribution are not significantly different from the expected ratios and indicates that the gene for curved spine behaves as an autosomal, single-factor Mendelian character recessive to the normal.

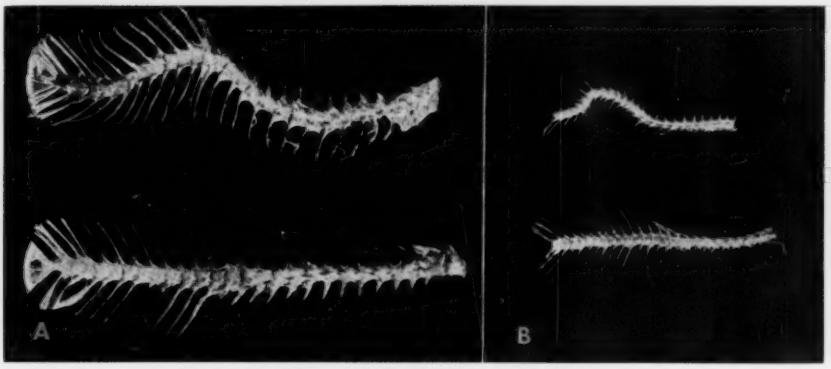
The marked curvature of the spine is readily apparent in the living fish at the time of birth of the fry and remains discernible throughout the life of the fish. Occasionally, mutant fish were born that appeared normal but these invariably developed the curved appearance within a few days after birth. Although all fishes were maintained under observation beyond the age of sexual maturity, no evidence for any sex-linked inheritance could be found.

TABLE I. Mineral composition of the spinal column from curved and normal *Xiphophorus* hybrids

	Curved	Normal	"t"	P
Spine, % body wt.	1.25 ± 0.04*(14)†	1.25 ± 0.03 (28)	0.00	N.S
Calcium, %	19.87 ± 0.35 (20)	17.68 ± 0.25 (20)	5.11	<0.01
Phosphorus, %	10.25 ± 0.14 (20)	9.22 ± 0.16 (20)	4.98	<0.01
Ca:P	1.94 ± 0.03 (20)	1.92 ± 0.07 (20)	0.57	N.S

$$*S.E = \left[\frac{\Sigma d^2}{n(n-1)}\right]^{1/2}$$

†The numbers in parentheses indicate the number of specimens analyzed.



CURVED AND NORMAL SPINES

Figure 19

A—Curved and normal spines from *Xiphophorus* sp. The spines are 3.3 cm in length. *B*—Curved and normal spines from *Lebistes*. The spines are 1.7 cm. in length.

In three separate trials over a five year period, double recessive male fish (*ScSc*) proved to be sterile in that no young were produced even after a six months mating period with subsequently proven fertile females. This sterility factor in the male required continuous backcross breeding trials in order to maintain the strain. Although the double recessive females (*ScSc*) became noticeably gravid when crossed with homozygous normal (*SnSn*) or heterozygous normal (*SnSc*) males, indications of decreased fertility in female fish was also apparent in many cases. In mutant females, broods rarely contained more than 20 young when 30-50 young were expected. Other signs of infertility were evident by the appearance of ova, immature broods, non-viable broods, and extrusion of the ovary[10].

The chemical analyses (Table I) demonstrates that the spines from mutant swordtails contain a higher percentage of calcium and phosphorus than the spines from the wild strain but the Ca:P is not altered. The relationship between spine weight and eviscerated body weight is the same for both mutant and normal swordtails. These data indicate that mutant bone is more compact and may contain less organic matter than normal bone. Unfortunately, sufficient animals are not available for further study at this time.

TABLE II. Inheritance of spinal curvature in *Xiphophorus* sp.

Cross		Observed		Expected		
Male	Female	Straight	Curved	Straight	Curved	χ^{2*}
SnSn × *ScSc*		113	0	113	0	___
SnSc × *SnSc*		133	52	139	46	0.95
SnSc × *ScSc*		70	91	80	81	2.74
ScSc × *ScSc*		Sterile				

*For one degree of freedom χ^2 values of less than 3.84 are not significant.

Although the inheritance of spinal curvature in swordtails behaves as a single-factor Mendelian recessive similar to that previously found in *Lebistes*[7], the chemical data indicate that the two mutations are not identical. In *Lebistes* the mutant spine is heavier in relation to body weight and contains more calcium than the normal spine[9]. On the other hand, the phosphorus concentration is the same for mutant and normal *Lebistes* thus resulting in a significantly different Ca:P. Other studies in *Lebistes* with radioactive nuclides indicate that the mutation involves a specific alteration of calcium metabolism[11]. It would appear, therefore, that a variety of genes are responsible for the formation of bone and skeletal structures and mutations of different gene loci result in the expression of phenotypes that are visually similar.

Four other strains of swordtails with spinal deformities were studied but these were not hereditary. Since our last communication, additional fishes with various vertebral abnormalities have been observed in the following species: *Betta splendons* (Siamese fighting fish); *Corydoras aeneus* (Catfish); *Anoptichthys jordani* (Blind cave fish); *Mollienisia latipinna* (Mollie); *Barbus semifasciolatus* (Golden barb); *Barbus tetrazona, Barbus conchonius* (Rosy barb); *Hyphessobrycon serpae,* and *Trichogaster leeri* (Pearl gourami). The frequency of appearance of spinal curvature in tropical fish culture indicates that the gene loci governing bone development and mineral metabolism are easily mutable.

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Plant Breeding and Cytogenetics

(*Continued from page 237*)

charged with the responsibility for plant exploration and introduction. It is equally important to see that no germplasm that may have potential value for the future is lost or discarded. Fortunately, this problem is commencing to receive the attention it deserves by scientists and legislative bodies.

It is surprising to find a book devoted to plant breeding that almost completely ignores the commonly used statistical methods, except for a list of references. Statistics are important tools in the plant breeder's arsenal, and some knowledge of statistical techniques is essential to the plant breeder, if for no other reason than to read intelligently the voluminous literature on the subject. The author probably feels that statistics should be given as a fully independent course in preference to including a superficial smattering of this discipline in a plant breeding text. Actually, there is considerable merit to this position.

Unlike some plant breeders, Professor Elliott is convinced that the breeder's responsibility does not end when he has turned over the product of his labor to the seed trade or to other commercial outlets for increase and distribution. To bolster this argument, he has devoted an entire chapter to release and post-release problems, discussing such matters as release procedure, crop improvement associations, deterioration of varieties, seed certification, etc.

There are about 1,000 literature references, critically selected to document the text; some of them are suited to serve as sources for advanced reading. The appropriate references are gathered together at the end of each chapter. For a textbook this plan has some advantages over the conventional arrangement of placing the bibliography in the rear of the book. The index is adequate and unusually accurate. The book is neatly composed, and printed on good quality paper; typographical errors are extremely rare. The illustrations are good, particularly the diagrams and charts, but the reproduction of some of the photographs is not as sharp as one might expect. One figure (7-2, pg. 204) is reproduced on such a small scale that its value is questionable. These are minor issues, and do not detract from an otherwise interesting, well written, and stimulating presentation.

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RED DANE AND MILKING SHORTHORN CROSSBREDS

Compared with Milking Shorthorns

A. C. Cook and W. M. Dawson*

IN a research study for the improvement of dual-purpose cattle in the production of both beef and milk at the Agricultural Research Center, Beltsville, Maryland, the United States Department of Agriculture in 1937 began a breeding experiment with a Red Dane bull, imported in dam from Denmark, and crossed with females in the Milking Shorthorn herd. The daughters of this cross were then mated to Milking Shorthorn bulls. The three kinds of progeny (purebred Milking Shorthorns, ½ Red Dane × ½ Milking Shorthorn, and ¾ Milking Shorthorn × ¼ Red Dane) received the same care and similar feed in order to have a comparison between them for the production of both beef and milk. All calves nursed their dams for the first three days and were then fed milk, grain, and hay individually to a weight of 500 pounds. Heifers were fed individually mixed grain and alfalfa hay from 500 to 700 pounds and turned in the breeding herd. They were bred at approximately 18 months of age and tested for milk production for 305 days of their first lactation. Male calves were castrated and fed a fattening ration to 900 pounds and slaughtered. Sixteen head of first-cross progeny (½ Red Dane × ½ Milking Shorthorn) and 18 head of backcross progeny (¾ Milking Shorthorn × ¼ Red Dane) were born in the herd. Photographs of represenative animals are shown in Figure 20.

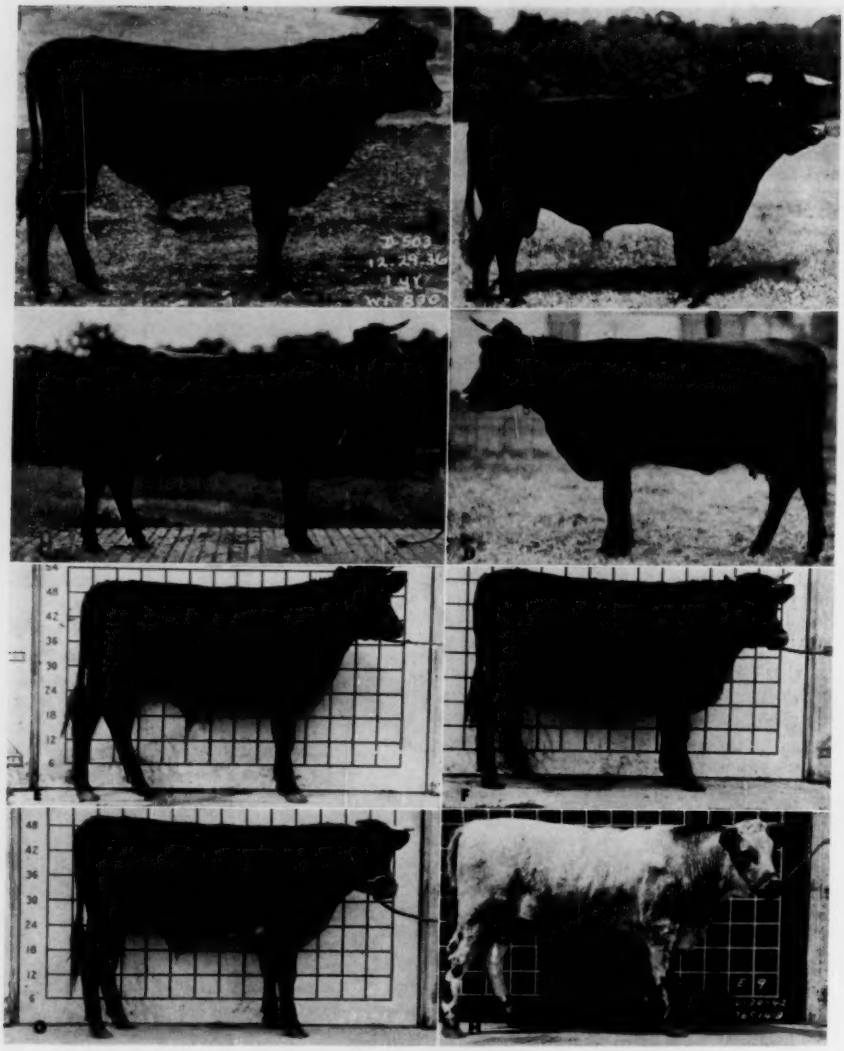
While the main objective of this study was efficiency of production, the coat color and color of muzzle were also recorded. Of the 16 head of first-cross progeny, nine were red and seven were dark roan. The dams of three of these roans were white, two were red and two were roan. Only one of the first-cross progeny had a clear (flesh-colored) muzzle, five were black and the other 10 had sooty (traces of black) colored muzzles.

In order to have the factor of years comparable in the study of beef production by the three groups of steers, 21 head of purebred Milking Shorthorn steers born in the herd during the same period as the crossbreds were used in the comparison. Three head of crossbred Red Dane × Milking Shorthorn steers and five head of backcross steers of ¾ Milking Shorthorn × ¼ Red Dane breeding and the 21 head of purebred Milking Shorthorn steers were fed individually to a weight of approximately 900 pounds and slaughtered under the regular record of performance procedure used at Beltsville.

Average Performance

The average performance of these three groups of steers is given in Table I. Although the first-cross steers, on the average, were heavier at birth, the backcross steers made approximately the same daily gain in the period from birth to 500 pounds. The Milking Shorthorn steers gained significantly less per day during this period. Neither in average daily gain nor in efficiency of gain (pounds of gain per 100 pounds of total digestible nutrients consumed) was there, statistically, a significant difference between the three types of steers. However, the differences found were in favor of the crossbreds. In total time to reach the slaughter weight, days from birth to 900 pounds, there were significant differences between the groups.

*U. S. Department of Agriculture, Washington, D. C. The authors wish to acknowledge the assistance of the late W. H. Black, formerly in charge of Beef and Dual-Purpose Cattle Investigations; R. L. Hiner, acting head of Meat Quality Laboratory; and Ralph Kennedy, Agricultural Aid, Agricultural Research Center, Beltsville, Md.; and E. B. Krantz, formerly in charge United States Morgan Horse Farm, Middlebury, Vermont.



RED DANE AND MILKING SHORTHORN CROSSBREDS

Figure 20

A—Red Dane bull D503 at one year of age used to sire first cross. 830 lbs. *B*—Milking Shorthorn bull MO45 at seven years of age used to sire backcross. *C*—1/2 Red Dane × 1/2 Milking Shorthorn cow 82X at three years of age after first lacatation. *D*—3/4 Milking Shorthorn × 1/4 Red Dane cow X92 at three years and two months of age. *E*—1/2 Red Dane × 1/2 Milking Shorthorn steer at 15 months of age. *F*—3/4 Milking Shorthorn × 1/4 Red Dane steer at 16 months of age. *G*—Milking Shorthorn steer D67 at 17 months of age. *H*—Milking Shorthorn steer E9 at 17 months of age.

Feeder, slaughter, and carcass grades were obtained in terms of the United States Department of Agriculture grade standard used prior to December 29, 1950[1]. No feed was given the animals for 24 hours previous to slaughter but they had access to fresh water. The dressing percent was based on slaughter weight (taken just prior to slaughter) and the chilled carcass weight. The differences in dressing percentage were on the border line of significance.

Numbers of steers in the two crossbred groups are too small to draw definite conclusions on the beef production part of the experiment. However, there appeared to be a tendency in these data for the first-cross and backcross steers to pe slightly superior to the Milking Shorthorns in rate of growth from birth to 500 pounds and during the fattening period from 500 to 900 pounds. In carcass quality the purebred Milking Shorthorn steers were slightly superior to the other two groups. This might be expected as the Red Dane cattle are more of a dairy type and did not carry the finish that is characteristic of dual-purpose cattle.

It was not possible to test most of the Milking Shorthorn dams and the crossbred progeny for milk and butterfat production at the same place. Thus nine of the ten Milking Shorthorn dams were milked at Middlebury, Vermont, while all the crossbreds were milked at Beltsville, Maryland. Although the animals were handled in a similar manner there may be differences due to location in the comparison. Only 10 crossbred daughters of Milking Shorthorn dams and only four head of backcross (¾ Milking Shorthorn × ¼ Red Dane) daughters had a complete production record for one lactation. The average production of the 10 head of purebred Milking Shorthorns for their first lactation was slightly less, 6,044 pounds of milk and 249 pounds of butterfat, than that of their first-cross daughters, 6,460 pounds of milk and 261 pounds of butterfat, but not significantly so (Table II). This was true both for their actual production and for their production expressed as a fat-corrected mature equivalent (fat corrected milk = .4 × milk + 15 × butterfat; age correction factors taken from Kendrick[2]). The Red Dane sire of these crossbred daughters was out of a Red Dane cow that was imported as a bred heifer from Denmark. This cow has four lactation records with an average mature equivalent of 6,186 pounds of milk, 285 pounds of butterfat and a test of 4.61 percent. This

TABLE I. Performance of steers

	Milking Shorthorn	½ Red Dane × ½ Milking Shorthorn	¾ Milking Shorthorn × ¼ Red Dane
No. of steers	21	3	5
Birth weight, pounds	78.2	93.3	79.2
Average daily gain			
Birth to 500 pounds	1.69	1.81	1.85
500 to 900 pounds	1.73	1.95	1.86
Feed consumed in 500 to 900 lb. fattening period per cwt. gain			
Concentrates, pounds	640	585	624
Alfalfa hay, pounds	361	356	323
Days from birth to 900 pounds	487	428	453
Feeder grade (average)	Medium	Low medium	Medium
Slaughter grade (average)	Medium	Low medium	Medium
Carcass grade (average)	Medium	Low medium	Low medium
Dressing percent	59.2	55.9	58.4

TABLE II. Comparison of milk production

	Number cows	305 days, milked twice a day actual production, 1st lactation			Fat corrected mature equivalent milk
		Milk	Test	Fat	
		(lb.)	(%)	(lb.)	(lb.)
Milking Shorthorn	10	6,044	4.12	249	8,120
½ Red Dane × ½ Milking Shorthorn	10	6,460	4.04	261	9,360
¾ Milking Shorthorn × ¼ Red Dane	4	6,226	3.84	239	9,173

is for lactations of 300 days and twice a day milking. Her first two lactations were made at Waseca, Minnesota, and averaged 5,667 pounds of milk, 259 pounds of butterfat, and tested 4.56 percent, while her last two records were at Beltsville, Maryland, where she averaged 6,705 pounds of milk, 312 pounds of butterfat, and a test of 4.65 percent. The maternal granddam of the sire gave 7,582 kilograms of milk and 336 kilograms of butterfat in Denmark in her third lactation.

The four backcross cows (¾ Milking Shorthorn × ¼ Red Dane) produced an average of 6,226 pounds of milk and 239 pounds of butterfat in their first lactation, or when figured as fat corrected mature equivalent 9,173 pounds of milk (Table II). This is slightly less than the average for the 10 first-cross cows. The Milking Shorthorn sire of three of these backcross cows was a proven sire out of a Milking Shorthorn cow with a record of 10,216 pounds of milk in 315 days in England with her first calf. Two of the backcross cows were out of one of the poorest first-cross cows. Therefore, the sample of backcross cows may not be very representative.

Conclusions

Although it is difficult to draw conclusions from this experiment because of the small size of the sample, large individual variations and possibility of differences due to location in the milk records it does seem desirable to place the results on record. If one assumes there is little, if any, bias due to possible location differences and that the sample of 10 Milking Shorthorn dams and their 10 first-cross daughters is representative there appears to be a trend towards an increase in milk production by the crossbred females. The data also appear to indicate a trend towards a greater daily gain, and a more rapid growth from birth to weaning and from weaning to slaughter for the crossbred steers with a tendency for lower carcass quality and dressing percentage in the crossbred steer progeny. These results are, in general, similar to those found by the United States Department of Agriculture from crossing Red Dane and Red Poll cattle[3].

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A NOTE ON CHINESE MARRIAGE CUSTOMS AND EUGENICS

On a recent examination, I asked my students to write an essay on some aspect of the eugenic problem. One of my students, Ng Chee Ping, wrote a paragraph which seems to be worthy of repetition. I have paraphrased it somewhat, as he still has difficulty with the English language. In substance, however, Ng wrote:

"In China, under the old-fashioned marriage customs, parents of an engaged couple usually investigated the health conditions of the other family for three generations back (i.e., parents, grandparents, and great-grandparents). While this was not consciously done for eugenic reasons, it was nonetheless a good method for eliminating undesirable genes. Modern marriage, however, is not controlled by the parents."

Few among us would care to revert to a system of parental control of marriage, yet it seems quite obvious that such a thorough investigation of the family background of a potential husband or wife should reduce some of the hazards of marriage. It could do nothing to prevent the tragedy of a defective child due to new mutation nor to recombination of recessives which had been hidden in the heterozygous state for many generations. But it would forewarn people of dominant defects and of those recessive defects which had appeared in the families concerned in the recent past. It would be most desirable to impress upon young people and their counsellors the importance of such an investigation of family background before marriage. Then, if investigation should turn up any seriously defective condition, genetic counselling could be sought *before* marriage, and at least some tragic problems could be avoided.

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THE NUCLEOLAR CHROMOSOMES OF ALLIUM CEPA

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A single pair of satellite-bearing chromosomes has been described characteristic of the somatic complement of *Allium cepa* by many workers [5,6,7,11]. In addition, only one or two nucleoli per nucleus have been found in most studies of this plant which has been extensively used in investigations of chromosome morphology. Mensinkai[9] reported finding nucleoli associated with two pairs of chromosomes but in somatic cells the second pair of satellites were not of the usual type if present at all. In a recent study of root tip squashes, the author observed three and four chromosomes with typical satellites, their presence being correlated with an increase in nucleolar number. Characteristic differences between varieties were observed as well. As the species has been subjected to cytological examination by a number of investigators over a period of many years it seems of interest to briefly describe and illustrate these findings.

Material and Methods

Three commercial varieties of *Allium cepa* viz. Early Yellow Globe, Southport White Globe and Red Weatherfield were studied. The bulbs were rooted in the usual manner and the root fixed in acetic-alcohol following pretreatment for one hour in a saturated solution of d — bromonaphthalene. A longer period than one hour was found to cause excessive contraction. They were stained according to the Feulgen procedure and squashed in aceto-carmine. Permanent preparations were obtained by the technique of Bhaduri and Ghosh[2]. The method of Bhaduri[1] was used for differential staining of nucleoi.

Photographs were taken using a Bausch and Lomb K camera at an initial enlargement of 1200 ×.

SOMATIC CHROMOSOMES AND NUCLEOLI

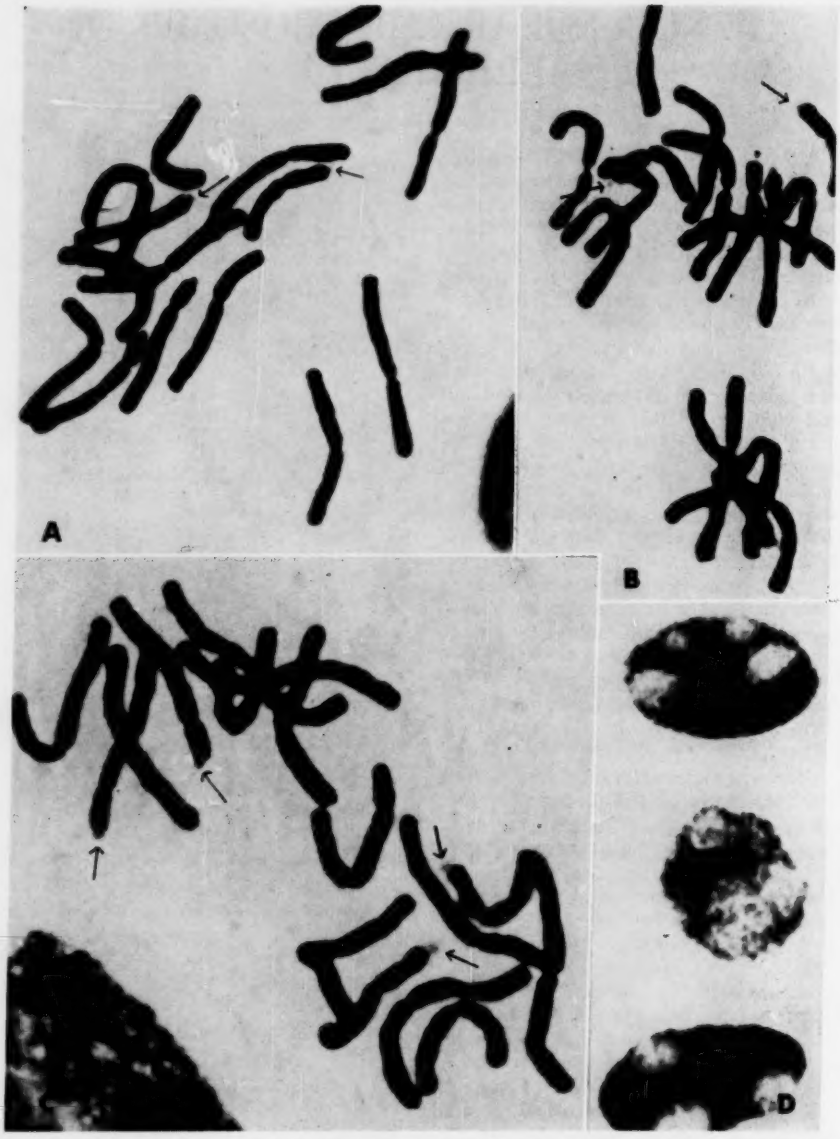
Figure 21

A and *B*—Satellites on the submedian chromosome are indicated by arrows. *C*—Nuclei showing one and two nucleoli (*A-B*—× 950, *C*—× 1680).

Results and Discussion

Study of the mitotic plates of the yellow variety revealed in addition to the previously illustrated satellited chromosomes, a second pair characterized by submedian centromeres and distinct satellites, (Figure 22*A-C*). The latter are smaller and less deeply stained than those of the long pair with subterminal centric constrictions. The red and white varieties consistently had a single representative of the

*Department of Genetics, University of California, Berkeley. Grateful acknowledgment is made to Dr. D. R. Cameron for suggestions and for reading the manuscript. Thanks are also due to Dr. Glenn Davis, Department of Vegetable Crops, University of California, Davis, for identifying the varieties.



SOMATIC CHROMOSOMES AND NUCLEOLI OF *A. CEPA* WITH FOUR SATELLITES

Figure 22

A and *B*—Arrows indicate the satellites on the submedian pair. *C*—All four satellites are designated. *D*—Nuclei of root tip cells showing three and four nucleoli. (*A*—× 2160, *B*—× 1920, *C*—× 2400, *D*—× 1800)

submedian type, (Figure 21*A-B*). While the possibility of the presence of a second chromosome of this type is not excluded only one was observed in studying many cells.

The relationship between satellite chromosomes and nucleoli[3,4] led to a study of the latter in the three varieties. While the number is, as usual, variable as a result of fusion there was a consistent difference in the maximum number present. The yellow variety was shown to have a maximum of four and a modal class of three (Figure 22*D*). Where three satellite chromosomes were regularly seen, the number of nucleoli was one or two, again depending on whether fusion had occurred (Figure 21*C*). The failure of the third satellite chromosome to form a nucleolus may be attributed to its inability to compete with two stronger nucleolar organizers. When present in duplicate this inability is overcome and one or two nucleoli are formed. Such competition has been described by Navashin[10] and McClintock[8].

Mensinkai[9] investigated the meiotic and mitotic chromosomes of two varieties of *Allium cepa*. His drawings show two pairs of chromosomes associated with nucleoli at diakinesis. However, the chromosomes concerned are clearly different morphologically from the satellite chromosomes of the present study.

The explanation for the difference in chromosome morphology described here is conjectural. California varieties such as those studied have apparently been isolated from those used by earlier workers for a long period of time. During this period it is conceivable that minor structural alterations have led to the transfer of the satellite of the subterminal chromosome pair to non-homologous submedian chromosomes. Subsequent hybridization for varietal improvement could then bring the two types together. Since the onion is largely propagated vegetatively, various combinations of 2, 3 and 4 satellites could be maintained over a considerable period of time. It should be of interest to study more material of additional varieties in this regard.

Summary

The presence of three or four satellite-bearing chromosomes in commercial varieties of *Allium cepa* is described in contrast to the results obtained by many previous investigators. In each instance they are shown to be associated with nucleoli, a maximum number of four where four satellite chromosomes are present but only two in the three-satellite forms.

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Index to Plant Chromosome Numbers

THE second issue of the Index to Plant Chromosome Numbers, compiled from nearly 300 journals published in 1957, is now ready for distribution. There are around 2,000 listings of original chromosome counts from the entire plant kingdom and a bibliography of 196 papers from which the listings were taken. Preparation of the Index has been supported in part by a grant from the National Science Foundation of the U.S.A. The price of each issue is $1. Orders for subscriptions may be sent to:

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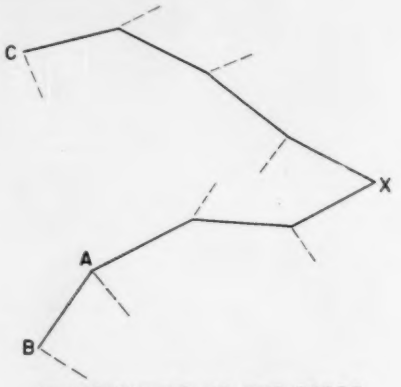
DODDLER CATTLE

An Inherited Congenital Nervous Disorder in Hereford Cattle

J. W. High, C. M. Kincaid and H. J. Smith*

IN the spring of 1952 a breeding program was initiated at the University of Tennessee Tobacco Experiment Station to develop a line of cattle around a progeny-tested young bull (bull A). The original cows in the herd were primarily from one source and unrelated to bull A. In the spring of 1954 nine daughters of bull A were mated to his son (bull B). In addition, bull B was mated to nine of the original cows and seven cows (all unrelated to him) that were bought in Texas. The cows in the breeding herd ranged from yearlings to 10 years of age.

The progeny produced from the original and Texas cows were all phenotypically normal. However, from the half-sib matings a calf exhibiting an extreme nervous disorder was produced. This calf—commonly referred to as a "doddler"—was characterized by extreme muscle spasms, convulsions, nystagmus and dilation of the eyes. Complete incoordination and difficult respiration were evident. The clonic movements were continual and both the body and extremities of the animal were affected. Although continual, the movements became less violent when the calf was allowed solitude. Upon stimulation from noise or bodily contact the movements would become much more pronounced. Another very noticeable characteristic was the animal's eyes. They were dilated and rolled around uncontrollably and continually, almost as if they were unattached. The calf, which was never able to stand, was killed at approximately five days of age.



RELATIONSHIP OF THE BULLS

Figure 23

The diagram above shows the relationship of bulls A, B and C.

Post mortem findings

No significant gross lesions were found other than rather severe congestion of the vessels of the pia mater. There was no external evidence of hydrocephalus. Histopathology of the brain revealed calcification of multiple neurons and small vessels in the brain stem and cerebellum. This was diagnosed as a probable sequel to degenerative changes that caused the nervous symptoms. The nervous disorder was present at birth.

In the spring of 1955, due to occurrences of dwarfism in the line, a bull from a different herd (bull C) was used. Bulls A and C trace to a common ancestor three and four generations removed, respectively. A diagram of the relationship between the three bulls is given in Figure 23.

Bull C was mated to nineteen daughters of bull A. Eight of these daughters had been mated to bull B the previous year. Bull C was also mated to eight of the original cows and to three Texas cows all of which were unrelated to him. The cows in the breeding herd ranged from two to 11 years of age. The prog-

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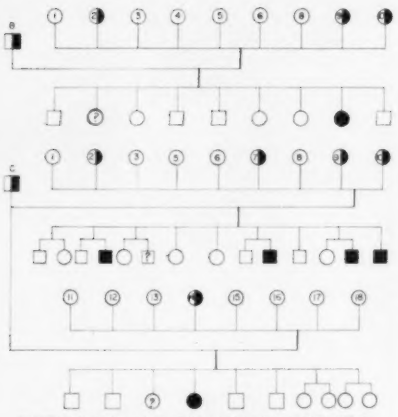

PEDIGREE OF DODDLER CALVES

Figure 24

All the females (1-18) in the diagram mated to bulls B and C are daughters of bull A. The progeny of these matings are shown just beneath their dams. Where two progeny are shown under a cow mated to bull C, the calf on the left is the '56 progeny and the calf on the right is the '57 progeny. The single calves from matings to bull C are all '56 progeny. "Doddler" calves are shaded solid and their dams half shaded. Calves with a question mark were born dead. Females 1, 2, 3, etc. are the same in both matings.

eny of the original and Texas cows were all normal phenotypically. The daughters of bull A produced two "doddlers." These "doddlers" exhibited the same characteristics as those described previously.

Seven daughters of bull A that had been mated to bull C in 1955 were again mated to him in the spring of 1956. Also mated to him were 15 of the original cows and one Texas cow. The cows in the breeding herd ranged from three to 12 years of age. The progeny of the latter matings were all normal phenotypically; however, three "doddlers" were produced by the daughters of bull A. Three calves were born dead (one each year) out of daughters of bull A (see Figure 24). No autopsies were made on these calves. It was impossible to determine from their external features if they were or were not "doddlers."

Review of the Literature

Several examples of hereditary nervous disorders have been reported in mammals. An inherited "waltzer" type of nervous disorder was reported in mice[5], rats[1], and rabbits[2]. Upon stimulation, animals with this defect waltzed or moved around in a small circle. Snell[10] observed three non-lethal "shaker" mutants in mice. Two other inherited neurological disorders in mice, "trembler" and "reeler", were reported by Falconer[4]. "Shaker" and "jittery", two nervous defects in chickens, were observed by Scott *et al.*[9] and Godfrey *et al.*[6], respectively. Muscular spasms in the "trembler" became so violent that afflicted animals were unable to stand beyond the age of 14 weeks. "Jittery" is characterized by a tendency of the head to be retracted over the back, accompanied by a rapid sidewise vibration. Both authors submitted data that indicate a sex-linked recessive gene is responsible for each condition.

Lush[8] described a non-lethal hereditary nervous condition in goats. Affected animals would become perfectly rigid for a period of 10 to 20 seconds when suddenly excited. Congenital palsy was observed in guinea pigs by Cole and Ibsen[3]. This condition was inherited as a lethal recessive.

Occurrence of hereditary congenital lethal spasms in Jersey cattle was reported by Gregory *et al.*[7]. Afflicted calves exhibited continual intermittent spasmodic movements of the head and neck, usually in a vertical plane. When the animals were forced to stand, spasms in both front and hind legs developed. Noise and shock neither initiated nor intensified the spasms.

Although the spastic condition in Hereford cattle is not homologous to any of the conditions cited, it is more closely related to the last mentioned. Both conditions are apparently congenital, lethal, and caused by a recessive gene. Here much of the resemblance ends. The nervous symptoms of the "doddler" differ from that reported in Jersey cattle in several respects. The body and extremities of the "doddler" are affected. Upon stimulation, the clonic movements become more violent. Up to two weeks of age affected calves were not able to stand even when assisted.

Evidence of Inheritance

At the time this study was made the daughters of bull A had produced six "doddler" and 24 phenotypically normal calves* from matings to bulls B and C over a three year period. During the same period of time and in the same herd, 43 calves out of cows unrelated to either bulls A, B, or C, were sired by bulls B and C. All 43 calves were normal pheno-

*Does not include three calves dead at birth.

typically with respect to the nervous condition described. The frequent occurrence of the defect in the former matings and the absence of the defect in matings of the same bulls to unrelated cows suggests that the defect is inherited.

Table I gives the distribution of calves produced by daughters of bull A by year and sire. As shown in Figure 24, both male and female "doddlers" have occurred. The ratio of normal to "doddler" calves for the years 1955 and 1956 strongly suggests a monofactorial recessive as the mode of inheritance (assuming bulls A, B, and C are carriers). If the hypothesis of a monofactorial recessive is assumed, the observed (7:1) ratio of normal to "doddler" calves for these two years fits the expected ratio perfectly, since one-half the daughters of bull A would be expected on the average to be carriers of the defect. Pooling the three years' progeny (as one observation), the observed ratio would fit the hypothesis of a monofactorial recessive.

The numbers are too small to establish the exact mode of inheritance. However, the number and distribution of defective calves appearing in the different matings, the sexes represented and the helplessness of afflicted animals suggests the "doddler" is inherited as a lethal monofactorial autosomal recessive.

Discussion

As shown in the diagram (Figure 23) bull B was a son of bull A. Bull A and bull C trace to a common ancestor three and four generations removed, respectively. However, if the "doddler" is inherited as indicated by the data presented, the defect could have come from still a more remote ancestor.

Bulls A and B proved to be carriers of the dwarf gene. Bull C has been mated to enough daughters of known carrier bulls to statistically (.05) prove him free of the dwarf gene. Radiographs of the lumbar vertebrae were made on four of the six "doddler" calves. Two of the calves had normal vertebraes and two had slightly abnormal vetebrae (B_2 classification). These data suggest that the "doddler" is not related to dwarfism.

A breeder has reported having 9 calves born with symptoms identical with those described in this article. These calves were produced over a two year period. According to the breeder, the dams of affected calves were all by the same sire. Two affected calves were produced from sire daughter matings. The remaining seven calves were sired by another bull. Plans are being made by the author to make a study of this herd.

After hearing reports of the "doddler" at the Greeneville station, the herdsman at Oak Ridge reported that a calf exhibiting a similar nervous condition had been born earlier at his station. This calf was sired by bull C.

The problem now appears to be of small magnitude. However, if this defect is inherited as indicated in the data presented, it could—if allowed to spread—become a serious problem. Certainly the defect is serious enough that occurrences of it should prompt breeders to cull against it rather than make further outcrosses to cover it up and thus spread it in the cattle population.

TABLE I. Distribution of calves produced by daughters of bull A

Year	Sire	No. of progeny*	No. of normal calves	No. of doddlers
1955	B	8	7	1
1956	C	16	14	2
1957	C	6	3	3
Total		30	24	6

*Three calves dead at birth omitted.

Summary

An extreme type of nervous disorder has been observed in Hereford cattle. Afflicted animals, commonly referred to as "doddlers," are characterized by extreme muscle spasms, convulsions, nystagmus and dilation of the eyes. Complete incoordination and difficult respiration were evident. The clonic movements were continual. Both the body and extremities of the animal were affected. The data presented give evidence the condition is inherited and from the distribution of affected calves the mode of inheritance is suggested.

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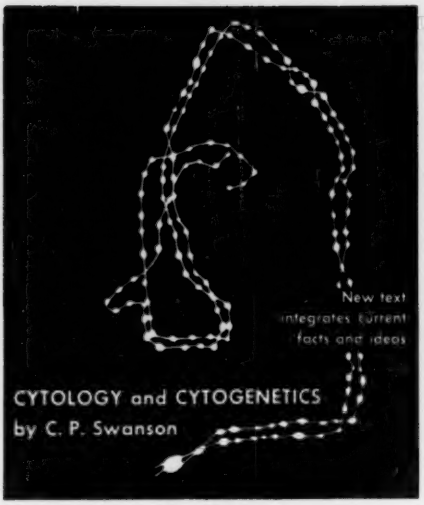

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